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PHYSIOLOGICAL REVIEWS

VOL. VI JULY, 1926 No. 3

THE BIOCHEMISTRY OF UREA

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In the organic history of life there occur certain substances which from the wideness of their distribution possess a general as well as a particular qualitative significance. Urea is one of these substances. Among mammals it is the principal non-protein constituent of the blood and urine. It is widely distributed in the animal kingdom, and, for the higher members, it constitutes the chief form in which nitrogen leaves the life-cycle. Among plants, urea is also of frequent occurrence, especially in the fungi; a diversity of distribution which suggests that it is a probable if not essential metabolite in the biochemistry of amino-nitrogen.

The study of urea has been greatly extended in the twenty-four years which have elapsed since the compilation of the monograph by Jacoby (153). Two extremely delicate and entirely independent methods of analysis have been introduced, depending on the specific decomposition of urea by the vegetable ureases (201), and on the formation of the sparingly soluble derivative of xanthydrol (92), (94), (98). The result of an extensive research on the chemistry of urea has resulted in a new structural formula which has several biochemical applications (299).

A SUMMARY OF THE EARLY HISTORY OF UREA. The ammoniacal fermentation of urine was well known among the ancients, and formed a principal source of ammonium carbonate, or "simple spirit of urine," which was widely used in medieval therapeutics, and was Van Helmont's remedy for "the jaundies." In the 16th century Nicholas Lemery (172) and later Scheele recognised the existence of an ammonia-forming substance in urine. This was ultimately separated, in 1773, by Rouelle, who extracted with hot alcohol the residue obtained by the careful evaporation of urine (258). In 1798, Fourcroy and Vaquelin

obtained urea nitrate in crystalline form by the addition of nitric acid to human urine and the urine of other animals. These notable investigators also showed that "this special material of the urine, which we now call urea . . . . . , gives rise to the carbonate of ammonia which replaces it in the decomposition of urine" (109).

The first accurate analysis of urea was made by Prout in 1824, and the chemical composition of natural urea was thus definitely established and recognised when Wöhler of Göttingen in 1828 (306) synthesised urea as the result of an attempt to prepare ammonium cyanate, and thus obtained a normal constituent of the urine, "without the aid of a kidney, an animal, a man, or a dog."

The subsequent history of urea may be followed along either of two paths according as the method of approach is chemical or biological. The chemistry of urea is discussed in Werner's monograph (299), to which the reader is referred. The physiology of urea formation and distribution as far as had been ascertained by the end of the last century is summarised in the articles by Schöndorff (267) and by Jacoby (155). An account of the natural distribution of urea will be found in the longer papers of Fosse (100), (105), (107).

THE DISTRIBUTION OF UREA IN NATURE. 1. ANIMALS. Investigations made in the last century were almost entirely confined to the detection of urea in higher animals, and although the methods of analysis were crude and uneconomical, they served to show the widespread occurrence of urea throughout the tissues (267). These observations have been revised and extended by the application of the urease method, the delicacy of which is determined only by the delicacy of the analytical methods for estimating ammonia, and the xanthydrol method, which provided an elegant gravimetric technique of almost equal delicacy but less specificity.

Urea in blood. Urea was first detected in blood by Prévost and Dumas in 1823 (247); since then it has been found to occur in the blood and tissue-fluids of all mammals, fish and amphibians investigated. It is present also, though in relatively lower concentrations, in the blood of birds, reptiles, and has been detected in the following invertebrates starfish, crayfish, lobster, shrimp, oyster, mussel, snail and some insects (95), (96), (100), (101), (106). Probably all animals which metabolise amino-acids are capable of producing urea.

The concentration of urea in human blood (89). Schwartz and McGill (271) have collected data from fifteen authors, which show a normal range of 11 to 25 mgm. urea nitrogen in 100 mls. of human blood. Folin

gives an average of 8 to 15 mgm. urea nitrogen in 100 mls., constituting 35 to 50 per cent of the total non-protein nitrogen (87), (90). McClean and Selling (192) consider that an upper limit of 22 mgm. urea nitrogen is within the normal, but this is above the values of most of the other investigators (159).

TABLE 1

The concentration of urea in the blood of mammals

ANIMAL	UREA N IN MILLIGRAMS PER 100 MLS.	UREA N AS PER CENT TOTAL NON-PROTEIN N	REFERENCE
Man (211 fasting subjects)	5.0–11.5		(271)
Man (whole blood)	11.5	35.2	(90) (87)
Man (plasma)	12.4	50.2	(90)
Man (serum)	11.0–26.0		(48)
Monkey	38.0	63.3	(89)
Dog (200 analyses)	5.0–42.0	31–53.9	(123)
(Average)	11.7	37.9	(123)
Dog	11.1–16.7		(263)
Dog (serum)	10.0–14.0		(202)
Cat (milk diet)	31.0		(216)
Cat (liver diet)	34.0	56.6	(84)
Cat (vegetable diet)	20.0	64.4	(84) (87)
Rabbit (average of 6)	13	41.9	(87)
Rabbit	6–15		(126) (12) (199) (200)
Sheep (average)	11		(263)
Sheep	13	46.4	(87)
Ox	14	58.3	(87)
Ox	13.6–15.3		(263) (113)
Cow	10.5–23.7		(263)
Horse	7.9–15		(263)
Horse	28	51.8	(87)
Pig	10–18.8		(263)
Pig	14	43.7	(87)
Rat	22	57.9	(207)
Guinea pig	11.9–24		(176)
Whale (average)	25.3		(235a)
Sea bear	18	21.9	(280a)

The distribution of urea in the blood. The results of Folin and Berglund (90) indicate that the corpuscles are freely permeable to urea, which would suggest a uniformity of distribution among the various components of the blood. This has been claimed for man (11), the cat (289), (243), and the rabbit (16), although a pathological increase in

blood urea may be more evident in the plasma than in the corpuscles (11). Folin and Berglund, however, find that the corpuscles are richer in urea than the plasma by over 2 mgm. per 100 mls., a result not in agreement with those of other workers (62), (281) including Polovinski and Auguste (245), who ascribe a lower corpuscular content of urea to

TABLE 2

The concentration of urea in the blood of non-mammals

ANIMAL	UREA N IN MILLIGRAMS PER 100 MLS.	UREA N AS PER CENT TOTAL NON-PROTEIN N	REFERENCE
Turkey	6.3		(263)
Duck	6.8		(263)
Duck	7.0	29.1	(87)
Goose	8.0	30.7	(87)
Hen	7.3		(263)
Chicken (average)	8.0	24.4	(87) (250)
Dove	5.2–8.7		(263)
Fish			
Teleosts (average)	6.0–18.2	65.0	(263)
Mackerel	10.0	11.5	(52)
Flounder	8.0	17.4	(52)
Shad	16.0	17.7	(52)
Eel	9.0	18.0	(52)
Goosefish	8.0	20.0	(52)
Elasmobranchs (average)	800.0	100.0	(29) (55)
Dog-fish	800.0	80.5	(55)
Skate	868.0	78.9	(55)
Sand-shark	1010.0	80.0	(55)
Shark	922.0–1246		(267)
Frog	7.5		(115)

Many of these figures are only of limited significance owing to lack of information about the total non-protein nitrogen of the samples, and the dietetic and other conditions. With few exceptions, the recorded values for mammalian blood area vary about a nitrogen average of 12 mgm. per 100 mls. of blood. In birds and in fishes the blood urea is about half this value, the undetermined nitrogen being remarkably high. The Elasmobranchs show exceptionally high concentrations of blood urea.

the presence of the stroma with its small capacity for solutes. The different analytical methods and animals employed may explain these discrepancies.

Pathological variations in the blood urea. This is outside the scope of the present review. The monograph by Feigl (72) incorporates the literature up to 1921, and should be consulted for details.

The distribution of urea in animal tissues. Urea is universally and fairly uniformly distributed throughout the organism, the concentrations being approximately that of the blood. Outstanding exceptions are the adipose tissue, which has a low urea content corresponding to

TABLE 3

The distribution of urea in the tissues of the dog (202)

TISSUE	UREA IN MILLIGRAMS PER 100 GRAMS MOIST TISSUE, OR 100 MLS. FLUID	
	Dog A	Dog B
Liver	23, 32	25
Limb muscle	25	18
Heart muscle	28, 30	20
Brain	28	20
Lung	31, 33	22
Spleen	28, 29	20
Pancreas	25, 26	18
Intestinal mucosa	29, 30	24
Omentum	5	6
Kidney	159, 183	221
Blood serum	29	21, 22
Urine	1640	
Bile	32	
Cerebro-spinal fluid	25	21

TABLE 4

The distribution of urea in the guinea pig, rabbit and hen (158)

TISSUE	UREA IN MILLIGRAMS PER 100 GRAMS MOIST TISSUE		
	Guinea pig (2)	Rabbit	Hen (4)
Blood	26, 26	60	6, 9, 12, 6
Liver	25, 26	65	15, 16
Heart	22, 23	38	11, 8, 10, 5
Lungs	23, 27	40	
Thigh	17, 19	32	6, 9, 11, 11
Abdomen	16, 31		
Breast			10, 10, 11, 11
Kidney	104, 50	155	10

its low content of water, and the renal tissue, which is high, owing to the presence of urine. These conditions of distribution have been found in the dog, guinea pig, rabbit, chicken, turtle and frog (271), (114), (158), (203). The most complete analyses by the newer methods are, at pres-

ent, those of Marshall and Davis for the dog (202), and Karr and Lewis for the guinea pig, rabbit and hen (158).

Allowing for the variation in the water content of the different tissues, results such as these indicate a tendency toward uniformity of distribution of urea in the organism. This might be anticipated from the high solubility, diffusibility and relative chemical inertness of urea (121), although the presence of a selective mechanism is claimed by Nonnenbruch (235) in determining the amount of urea in the secretions.

TABLE 5

The concentration of urea in the secretions

SECRETION	UREA N IN MILLIGRAMS PER 100 MLS.	AUTHORITY
Cerebro-spinal fluid:		
Human	23.3	(39)
	20.0–40.0	(48)
Dog	11.0	(202)
Saliva:		
Human	9.7–40.5	(56)
	12.4–19.4	(114)
Milk:		
Human	8.3–16.0	(53) (54)
Cow	17.5	(114)
	5.5–19.3	(219)
Goat	39.1	(219)
Gastric juice:		
Dog	13.6	(114)
Bile:		
Human	18.0–40.0	(46)
Dog	16.3	(114)
Ox	16.4	(113)
Frog	7.8	(115)

For example, the urea content of human cerebro-spinal fluid is lower than that of the blood by 10 to 15 per cent, and its variations follow those of the blood urea (245), (212). The urea content of saliva is about 10 per cent lower than that of the blood (266), and may be estimated as a substitute for blood urea estimations (131), but the method can hardly be trustworthy on account of the wide variation in the composition of mixed saliva (56).

THE EXCRETION OF UREA. Urea may leave the higher organism in three ways: by the kidneys, by the intestine, and by the skin. Of

these, the first is by far the most important, but in conditions of renal inefficiency the cutaneous excretion of urea may become considerable. Gad-Andresen (114) reports a concentration of 19.8 mgm. urea nitrogen in 100 mls. of ox sweat; and Benedict has found in man a cutaneous excretion of nitrogen amounting to 0.1 gram per diem. His paper should be consulted for a discussion of the literature (27).

Little appears to be known about the intestinal excretion of urea. Probably it is negligible in health. The readiness with which urea can be absorbed from the intestine (86), and the fact that many of the intestinal organisms are urealytic (170), (187), render it unlikely that urea would remain for long in the alimentary canal. Urea decomposition

TABLE 6

Daily output of urea in the urine of different races

RACE	UREA N IN GRAMS	TOTAL N IN GRAMS	$\frac{\text{UREA N}}{\text{TOTAL N}}$ %
Brahmin (vegetarian)	3.90	5.10	76.59
Hindoo (vegetarian)	4.75	5.97	79.60
Chinese	7.02	8.60	81.63
Tamil	7.67	9.63	82.09
European (tropical)	9.78	11.70	83.59
Malay	6.89	8.74	78.91
Eurasian	7.98	9.89	80.75
Bengali	6.08	7.57	80.37
Sikh	7.03	8.64	81.45
European standard	13.6	16.0	85.0

in the intestine may account for some of the ammonia of the portal blood (85), (18).

THE CONCENTRATION OF UREA IN URINE. Fourcroy and Vaquelin (109) were the first to show that urea is the principal nitrogenous compound present in the urine of higher animals. Modern analyses have confirmed this early observation as far as mammals are concerned, the outstanding exception being the camel (251), which has only a trace of urea in the urine. Since urine is an excretion its composition will vary continuously with the requirements of the organism, and consequently the concentration of urea in the urine may alter within very wide and, as yet, not absolutely defined limits. It cannot be reduced to a zero value by complete starvation, as the autolytic processes of the tissues afford a supply of its precursors as long as

the animal remains alive. Its maximum concentration depends on many factors, which will be considered later.

Under the usual circumstances of western civilization the urea concentration of adult human urine is 1.9 to 2.5 per cent, corresponding

TABLE 7

The concentration of urea in mammalian urine

ANIMAL	UREA N IN MILLIGRAMS PER 100 MLS.	UREA N AS PER CENT OF TOTAL N	AUTHORITY
Man:			
America	1390	87.5	(90)
France	1162	81.3	(8)
Germany	960	85.5	(114) (167)
Monkey		86.8	(146)
Tiger	3410	88.8	(112)
Leopard	3390	88.9	(112)
Hyena	3200	87.9	(112)
Dog		86.2	(174) (9)
Cat	1162		(113)
	1334		(113)
		62.5	(120)
Marten	2205	89.4	(112)
Weasel	986	87.1	(112)
Coyote	3226		(117)
Rabbit:			
Milk diet	204	89.5	(273)
Green diet	147	82.8	(273) (9)
Rat	2284	77.3	(207)
		77.1	(87)
Muskrat	708	92.5	(117)
Pig	2254		(43) (163)
Cow	490–1300	58.1–69.4	(179)
Ox	150–1500		(113)
Sheep	1270–1680	81.9–84.5	(179) (146a)
Goat	1650–3130	70.0–79.2	(179)
Horse	1426		(113)
	782– 805		(43)
Camel	Trace		(251)
Dolphin	1370–1514	78.4–80.9	(280a)

to a daily output of about 30 grams, and constituting 80 to 88 per cent of the total urinary nitrogen (82). This ratio may be raised to 90 per cent on a milk diet (167). In children the total urea output is, as might be expected, lower than in the adult, but the excretion relative to the

weight of the body is higher. The average proportion of total urinary nitrogen in the form of urea in children is 83.7 per cent (156). Race apart from diet appears to have no effect on urea excretion, as is shown by the following results obtained by Campbell (38).

Health has been maintained on a diet which gives a urinary nitrogen concentration as low as 261 mgm. per 100 mls., constituting 62 per cent

TABLE 8

The concentration of urea in non-mammalian urine

ANIMAL	UREA N IN MILLIGRAMS PER 100 MLS.	UREA N AS PER CENT OF TOTAL N	AUTHORITY
Alligator	13.3		(177)
Turtle	12.9–21.5	31.1–45.1	(177)
Python	12.0	3.34	(112)
Cockerel	28.0*		(207)
Goose fish	260.0	81.0	(52)
	339.0	80.7	(52)
Dog fish	120.0	14.4	(52)
	248.0	62.0	(52)

* Urea and NH_3.

TABLE 9

Miscellaneous occurrences of urea

SOURCE	UREA N IN MILLIGRAMS PER 100 MLS.	UREA N AS PER CENT OF TOTAL N	AUTHORITY
Allantoic fluid:			
Cow	19.0–37.0	12.6–38.4	(179)
Sheep	43.0–58.6	15.2–33.0	(179)
Amniotic fluid:			
Cow	11.6–12.0	42.8–63.0	(179)
Sheep	20.0–72.0	61.7–88.0	(179)
Peritoneal fluid:			
Whale	2.8		(235a)

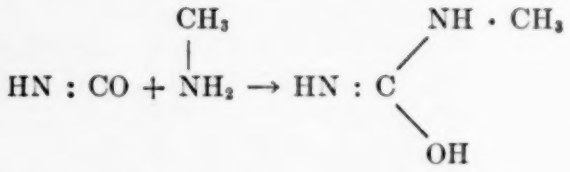
of the total urinary nitrogen (82). When the daily elimination of nitrogen is reduced from its normal value of 12 to 16 grams to such low values as 3 to 4 grams, only about 60 per cent represents urea. A fasting subject has been described (82) in which the urine contained 680 mgm. of urea per 100 mls., representing only 14.7 per cent of the total nitrogen; here it must be observed that 40 per cent of the total nitrogen was in the form of ammonia.

Benedict and Diefendorf report similar low values (28), also Robison, whose figures are amongst the lowest recorded for the human subject (256). All of these results support an early conclusion of Folin (82) that urea is the only nitrogenous constituent of human urine which suffers a relative as well as an absolute diminution with a diminution in total nitrogen metabolism.

Since the composition of urine is subject to such wide dietetic variations, it is important to note that the results of urea estimations may be entirely misleading unless expressed on the basis of the total nitrogen content over a definite period (82). For this reason many of the analytical data here summarised must be considered incomplete.

Urea as a solute. The uniformity of results obtained by different methods of analysis goes to indicate that urea exists in blood and in urine as a simple solute (163). This agrees with the fact that urea can be readily separated by dialysis from its complex solutions. The presence of colloids may, however, obscure some of the reactions of urea in a way that suggests the formation of unstable compounds (299). This may explain the belief held by Moor (217) that half of the supposed urea content of urine is not due to urea but to an unknown constituent, which he called "ureine," and claimed to have separated. "Ureine" has been found by Haskins (127) and others to be a mixture largely contaminated with urobilin.

Substituted ureas in the urine. Folin has shown the presence in human urine of traces of methyl-urea (83). This is probably formed by the interaction of methylamine and cyanic acid, in a manner similar to the formation of ethyl urea after the administration of ethylamine carbonate (265), (299).



THE OCCURRENCE OF UREA IN NATURE. 2. PLANTS. The important position occupied by urea in the animal kingdom diverted the attention of investigators from its possible occurrence in plants, until early in the present century Bamberger and Landsiedl (15) found urea to the extent of 3.5 per cent in the fungus *Lycoperdon Bovista*. This was confirmed (116) and shown to apply to other fungi (118), (150). Since then, Fosse (95) has shown the formation of urea in the leaves of spinach,

endive, turnip, carrot, potato, gourd, melon, and in the leaves and young plants of barley, maize, wheat, clover, beans and peas. It appears to be absent from the leaves of certain perennials, such as the mulberry, a fact utilised by Fosse to show the synthesis of urea by silkworms (96). While urea has so far only been found in small amounts in green plants (for example, 64 mgm. per 100 grams dried solids of the green pea), it attains to a much higher concentration in fungi (150), (151), though this may in part be due to absorption from the soil. The accumulation of urea in plants without the power of synthesising carbohydrates appears to be associated with a definite carbohydrate deficiency (151). The urea nucleus occurs in at least one important alkaloid, *galegine*, from *Galega officinalis*; this compound on hydrolysis forms urea and an amine (17).

Very little information is available as to the occurrence of urea among the microörganisms. "Urea nitrogen" has been determined in the broth cultures of twelve different species of bacteria (162), its significance is as yet unknown.

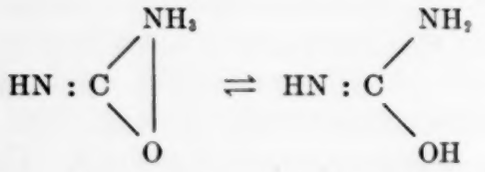
THE STRUCTURE OF UREA. The concept of urea as carbamide, $CO(NH_2)_2$, the diamide of carbonic acid, arose from a suggestion of Dumas (60); the actual term being first used by Regnault (252) in describing an attempt to obtain urea from carbonyl chloride and ammonia.

This particular synthesis was effected later by Natanson (225), and accepted as a direct proof of the carbamide constitution, which was further supported by Basarov's synthesis of urea by the action of heat on ammonium carbonate and carbamate under pressure (19). Later syntheses of urea (303), (133), (42) provided additional grounds for belief in the carbamide formula, which appeared to give an entirely adequate explanation of all these reactions.

The general decompositions of urea also proceeded apparently in accordance with the carbamide formula. Nitrous acid and alkaline hypohalogenites react with urea in a manner analagous to the way in which they react with compounds containing amino groups. Finally, the various biochemical syntheses of urea from ammonia and carbon dioxide were inspired by, and supported, the carbamide type of structure (265), (268).

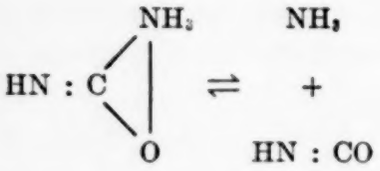
In 1912, Werner, as the result of a study of thiourea, was led to a close quantitative investigation of many of the familiar reactions of urea from which he came to the conclusion that urea is not carbamide, but

exists in neutral solution and in the solid state as a cyclic compound which can be opened up by acids or alkalies to form the unsymmetrical *iso*-carbamide:



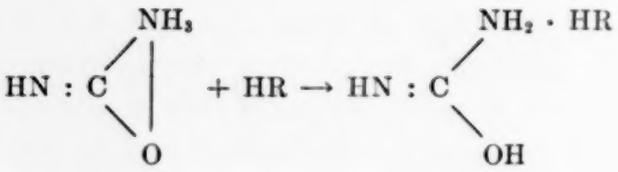
Urea (*cyclic form*) Urea (*open form*)

Urea is a derivative of ammonia and *keto* (or *iso*) cyanic acid, HN: CO, from which it can be formed by direct union, and into which it can be dissociated by heat (299) in a manner exactly comparable with the other salts of ammonia:



The amphotericity of urea is due to its capability of being changed from the cyclic to the open-form by both acids and bases:

(*a*)



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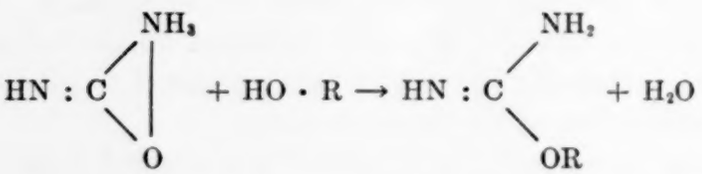
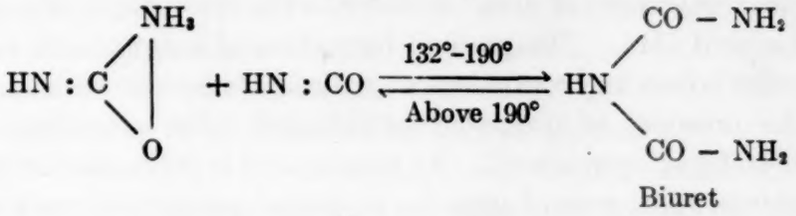


The cyclic formula has been applied by Werner to explain the various reactions of urea, of which the following are of sufficient biochemical interest to describe in detail.

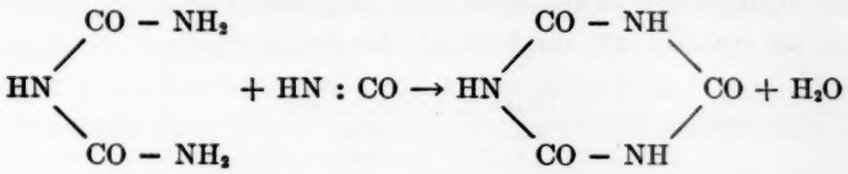
Decomposition of urea by heat. The first stage is the dissociation of urea at a temperature just above its melting point into ammonia and cyanic acid; the subsequent changes depend on the behavior of the highly reactive cyanic acid.

a. Formation of biuret by the action of cyanic acid on urea



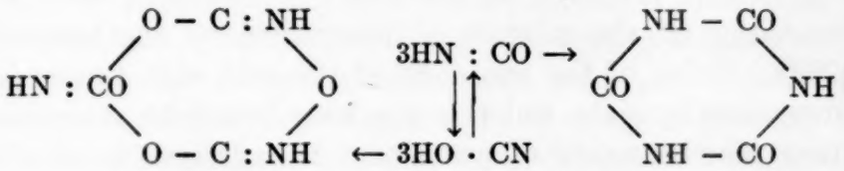
Biuret

b. Formation of ammelide by the action of cyanic acid on biuret



Ammelide

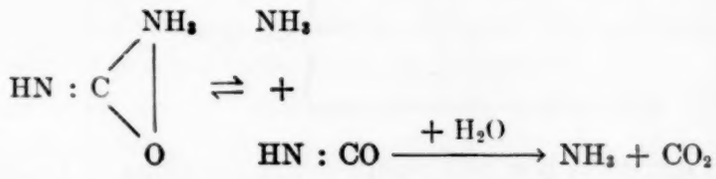
c. Formation of cyanuric acid and cyamelide by polymerisation of cyanic acid



Cyamelide
(Formed at low temperature)

Cyanuric acid
(Formed at high temperature)

DECOMPOSITION OF UREA IN AQUEOUS SOLUTIONS. *The "hydrolysis" of urea.* In *sterile* aqueous solutions urea is more stable than is usually recognised, and does not dissociate into ammonia and cyanic acid to any appreciable extent at temperatures much below 50°. This change, however, is readily brought about by acids or alkalies at high temperature, or by high temperature alone, or by the enzyme urease under zymolytic conditions (299), (66). The subsequent changes are determined by the agents causing the dissociation. The "hydrolysis" of urea is really the hydrolysis of the cyanic acid produced by dissociation of the urea:



It is important to observe that *dissociation* is used here in its original meaning, and is not synonymous with *ionisation.* In view of the con-

fusion through carelessness of the two terms, Davis has adopted the phrase "dearrangement of urea" to describe the resolution into ammonia and cyanic acid (51). The gradual formation of ammonia in solutions of urea under laboratory conditions at ordinary temperature is invariably due to the presence of urealytic moulds and other organisms (66).

The chemistry of cyanic acid. As cyanic acid is intimately concerned in the various syntheses of urea its principal properties must be considered briefly.

In the liquid state below zero, and in aqueous solution, cyanic acid exists as an equilibrium mixture of the two forms:

$$HO - CN \underset{\text{(Fall in temperature)}}{\overset{\text{(Rise in temperature)}}{\rightleftharpoons}} HN : CO$$

Enol-form, stable only at low temperatures. — Keto-form, stable at high temperatures.

The composition of the mixture is determined by the temperature (298), (299). Union of the enol-form of the acid with ammonia produces ammonium cyanate; union of the keto-form with ammonia produces urea. The behaviour of cyanic acid on hydrolysis is remarkable. In acid or neutral solution it is rapidly hydrolysed to ammonia and carbon dioxide, carbamic acid being formed as an intermediate stage (70):

$$HN : CO + H_2O \rightarrow \begin{matrix} NH_2 \\ | \\ CO \\ | \\ OH \end{matrix} \rightarrow \begin{matrix} NH_3 \\ + \\ CO_2 \end{matrix}$$

In alkaline solution or in presence of ammonium salts it is converted into urea:

$$\left.\begin{matrix} HN : CO + H_2O \rightarrow \begin{matrix} CO_2 \\ + \\ NH_3 \end{matrix} \\ HN : CO \longrightarrow \end{matrix}\right\} \rightarrow HN : C \begin{matrix} \diagup NH_3 \\ \diagdown\ | \\ \quad O \end{matrix}$$

General methods for the production of urea. Apart from the metabolic activities of living organisms, urea may be obtained by the following methods which either involve the employment of living tissues

or the use of bio-organic compounds under conditions approximating to those found in life:

1. Oxidation of proteins in alkaline solution.
2. Oxidation of amino-acids and their salts.
3. Deamination of amino-acids by hepatic perfusion.
4. Oxidation of ammonia in presence of hydrocarbon nuclei.
5. Dehydration of ammonium carbonate and carbamate by hepatic perfusion.
6. Dehydration of ammonium carbonate and carbamate by reversible zymolysis, electrolysis, and irradiation.
7. Direct union of ammonia and keto-cyanic acid.

These methods of preparation are not exclusive in their nature; several have reactions in common, and the last one represents the penultimate stage of all the preceding methods.

1. Formation of urea from proteins by oxidation. In 1842, Dumas and Cahours (61) suggested that urea was an oxidation product of nitrogenous metabolism. Béchamp (21) sought to confirm this experimentally, and claimed to have obtained urea by the oxidation of native proteins by means of potassium permanganate. A long controversy followed (277), (22), (279), (23), (181), (255), (186), (58), (59), (140), (157), (270), (141), (109), (1), (67), until the unequivocal results of Fosse (93), (94), (104) proved the correctness of Béchamp's early conclusion.

This controversy had its origin partly in the uncertainty of the methods formerly employed for the detection of small quantities of urea, but chiefly owing to the inability to appreciate the experimental conditions necessary for the production of urea. These are alkalinity and low temperature, otherwise the cyanic acid, which is the precursor of urea, is completely hydrolysed to carbon dioxide and ammonia (299).

Interest in the proteins as a source of urea gradually declined owing to the abandonment of the Voit and Pflüger theories of metabolism, and the recognition of the significance of amino-acids in nutrition. At the present time there is no reason to believe that urea is ever formed in the organism as the result of direct oxidation of tissue proteins. Among the simpler decomposition products, modern biochemistry has found sources of urea which are more direct and economical.

2. Formation of urea from amino-acids by oxidation. When amino-acids, and many other compounds containing the amino-group, are carefully oxidised in neutral or alkaline solution urea is formed (102), (104), (299), (68). Hofmeister, who was among the early investigators, concluded that this urea arose from the union of $-NH_2$ and $=CO$, and

that compounds free from nitrogen could give rise to urea if oxidised in presence of ammonia. Fosse, who confirmed and extended Hofmeister's work, showed that the quantity of urea formed by the oxidation of amino-acids and certain proteins could be greatly increased by incubating the solutions with ammonium salts after oxidation (103), (105), (106), (107).

Here cyanic acid is formed, but is fixed as an alkaline cyanate. On incubation with aqueous ammonium chloride urea is formed as a result of the isomeric transformation of ammonium cyanate. This work has been confirmed by Werner, who has obtained an 83 per cent yield of cyanic acid by the oxidation of glycine with sodium hypobromite (300).

3. Formation of urea from amino-acids by hepatic perfusion. Urea is formed when aerated solutions of amino-acids in blood or saline are perfused through the surviving mammalian liver. This has been shown to occur with glycine (154), alanine (183), leucine (259), (183), aspartic

TABLE 10 (104)

AMINO-ACID	UREA FORMED AFTER OXIDATION WITH $KMnO_4$	UREA FORMED AFTER INCUBATION OF OXIDATION PRODUCTS WITH NH_4Cl
Glycine	0.57 per cent	17.5 per cent
Asparagine	Trace	10.9 per cent

acid (183), asparagine (259) and serine (183); and confirms the earlier results of Embden, Salomon and Schmidt (63).

Ornithine, lysine, tyrosine, cystine, and taurine do not give rise to urea on hepatic perfusion (259), (73), which may account for the appearance of the last of these amino-acids in the bile.

Fiske and Karsner (79) found no increase in urea on perfusing the livers of the cat and the rabbit with homologous defibrinated blood containing up to 44 mgm. of glycine per 100 mls. They found, later (81), that increased oxygenation of the perfusion fluid increased the hepatic formation of urea from ammonium salts, and hence the negative result obtained on perfusion with amino-acids may be due to inadequate aeration. There is evidence, as will be seen further on, that the liver of the dog is more intimately concerned in urea production than the liver of the rabbit or the cat, and the varying results of different investigators may be due to the species differences in the experimental animals employed.

4. Formation of urea by the oxidation of ammonia. Hofmeister's

suggestion that urea may be produced by the oxidation of carbon compounds in presence of ammonia has been amply demonstrated by Fosse (93), (105), who has obtained cyanic acid, and hence urea, from the oxidation of ammoniacal solutions of alcohols, aldehydes, ketones, amides, amines, and carbohydrates. Werner (299) considers that the final stages in all these oxidation syntheses are:

$$CO + NH_3 + O \rightarrow H_2O + HN : CO$$

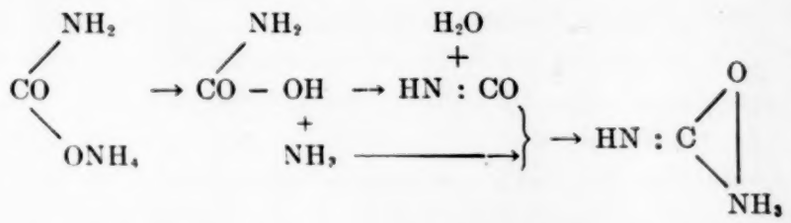
$$+ NH_3 \rightarrow HN : CO \cdot NH_3$$

5. Formation of urea by the dehydration of ammonium carbonate. This was first accomplished by Basarov (19), and has been repeated by many subsequent investigators (36), (215), (205), (74), (13), (166), including Lewis and Burrows, who have studied the equilibrium conditions (176).

The first stage in the transformation is the dehydration of the carbonate with the production of the carbamate. This occurs as an equilibrium reaction when ammonium carbonate is heated in a sealed tube or when it is dissoved in water to form a concentrated solution:

$$NH_4 - O - CO - O - NH_4 \rightleftharpoons NH_4 - O - CO - NH_2 + H_2O$$

Faurholt (65) has found under given conditions that the equilibrium mixture contains 80 per cent carbonate and 20 per cent carbamate. The subsequent change, generally supposed to be a direct dehydration of the carbamate as suggested by Dumas in 1830 (60), is now shown to be due to a dissociation of the carbamate with liberation of free carbamic acid, which is dehydrated to form keto-cyanic acid, the direct precursor of urea (299).



(Carbamate) *(Carbamic acid)* *(Cyanic acid)* *(Urea)*

The dehydration of carbamic acid does not proceed with the same readiness at low temperatures as is generally assumed. After twelve months' incubation in a sealed tube at 50° an 11.2 N solution of ammonium carbonate-carbamate equilibrium mixture yielded only 37

mgm. urea per 100 mls., corresponding to 0.03 per cent of the theoretical. Similar solutions kept from periods of one to two and a half years at ordinary laboratory temperature yielded no urea, or less than 0.1 mgm. per 100 mls. (301). Basarov's synthesis has been invoked by Schmiedeberg to explain the formation of urea in the animal body (265), and his work has received striking support from the classical experiments of Schröder (268), (269), who obtained a definite increase in urea by the perfusion of dogs' livers with dilute solutions of ammonium carbonate in blood. No such increase was observed with the kidneys and the limb muscles under similar experimental conditions. This has been confirmed and amplified by many later workers (261), (153), (183), (79), with the exception of Antonio (10), whose negative results may be explained by the observations of Fiske (80) on the necessity for adequate aeration of the perfusate.

This method of urea formation appears to be irreversible; even when the perfusion fluid contains 4 per cent of urea the liver continues to produce it (294), (155).

6. The formation of urea from ammonium carbonate and carbamate by reversion zymolysis, electrolysis and irradiation. Urea may be obtained by the action of soy and jack bean urease on concentrated solutions of ammonium carbonate and carbamate at temperatures of 40° to 60° (161), (195). Were urease a recognized constituent of animal tissues this reaction would offer a possible mode of origin of urea. But, so far, urease has only been demonstrated in the gastric mucosa of certain carnivora and herbivora (188),[1] where it may possibly have originated from infected food material since the enzyme is very widely distributed in nature (170), (187). Urea is also formed when concentrated solutions of ammonium carbonate and carbamate are electrolysed (213), (75), (76), or incubated at body temperatures with active charcoal (77), or irradiated with the quartz mercury-vapor lamp (71). The change in all these reactions probably involves a preliminary oxidation of the ammonia group, and a subsequent formation of cyanic acid.

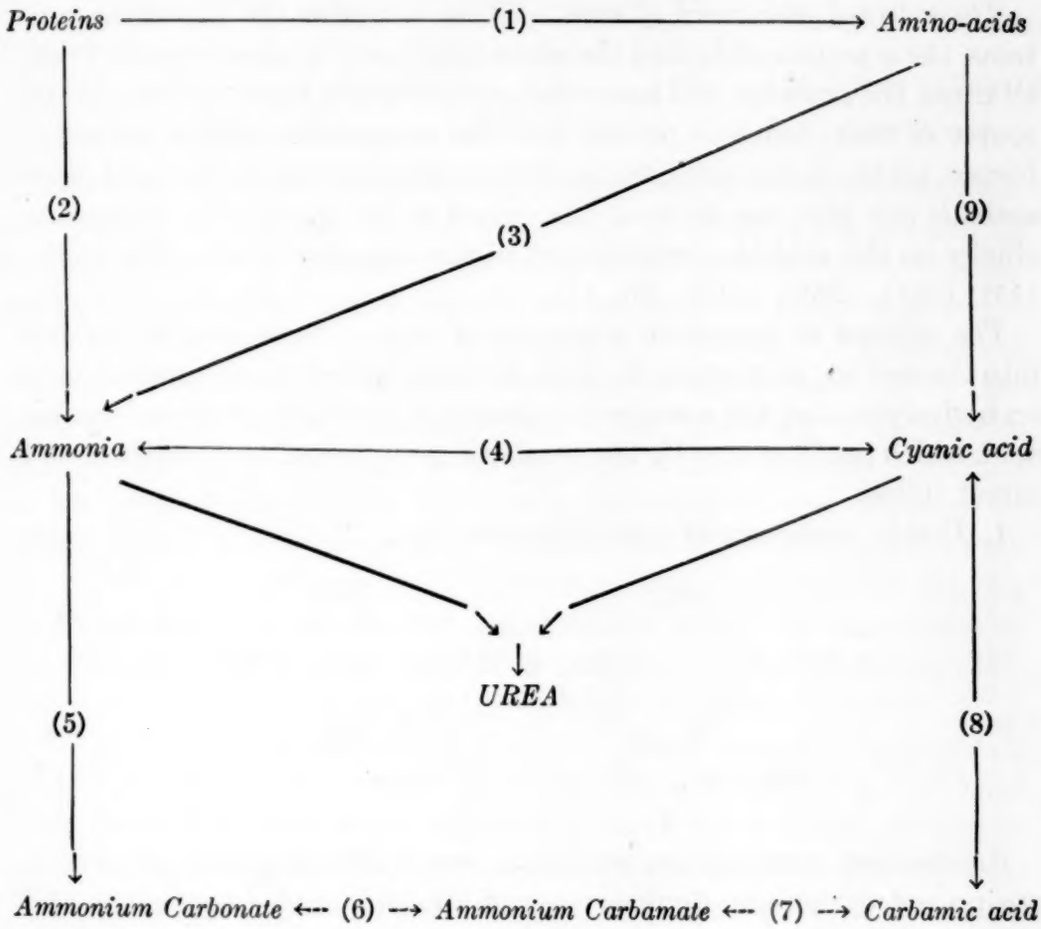
7. The formation of urea from ammonia and cyanic acid. When ammonia is added to a cold solution of cyanic acid it rapidly combines with the keto-form of the acid to produce urea. The enol-form of cyanic acid combines with ammonia to produce ammonium cyanate, which gradually reverts by dissociation and equilibration into the keto-form, and thus is ultimately converted into more urea.

[1] Urease has recently been found in the tissues of *Limulus*, but not in closely related crustaceans (172a).

$NH_3 + HO \cdot CN = NH_4 \cdot O \cdot CN$ (*Ammonium cyanate*)

$NH_3 + HN : CO = HN : C(NH_3)(O)$ (*Urea*)

GRAPHIC SUMMARY OF THE METHODS FOR OBTAINING UREA



Mechanism:

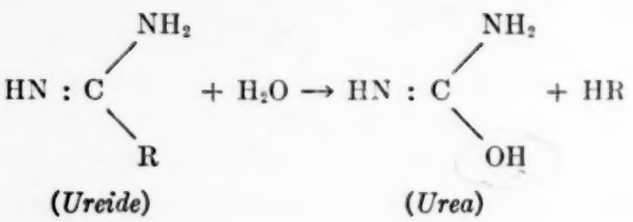
(1) = Hydrolysis.
(2), (3), (9) = Deamination.
(4) = Oxidation in presence of hydrocarbon nuclei.
(5) = Union with carbonic acid.
(6) = Hydration-dehydration equilibrium.
(7) = Dissociation.
(8) = Dehydration.

UREA FORMATION IN ANIMALS. Since urea is not a constituent of the diet its presence in the animal organism must be the result of a metabolic process. Consequently, two distinct classes of compounds are concerned: the *external* precursors of urea, which occur in the diet, and the *internal* or immediate precursors of urea, which occur in the tissues, but not in the normal diet. Furthermore, there is the chemical problem of the origin of the internal precursors and their conversion into urea, and the physiological problem of the location and control of urea formation.

The external precursors of urea. These comprise the digestible proteins, the α amino-acids, and the ammonium salts of most organic acids. Of these, the proteins, and hence the α amino-acids, represent the normal source of urea; but it is proved that the ammonium salts of carbonic, formic, acetic, lactic, and citric acid when administered to man and other animals can give rise to urea, the extent of the production depending chiefly on the acid-base equilibrium of the organism (260), (37), (125), (14), (287), (288), (111), (6), (7).

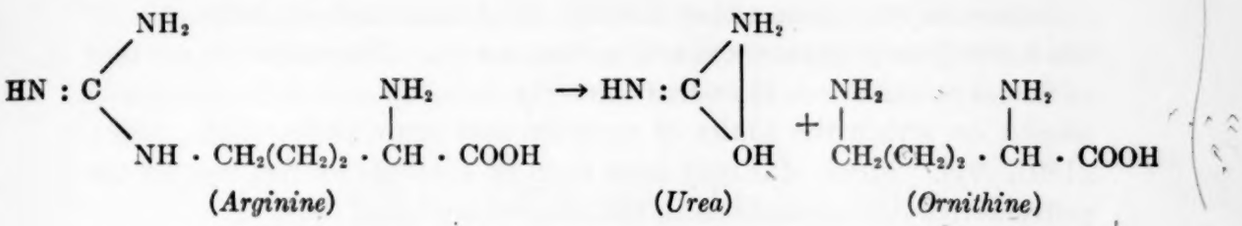
The internal or immediate precursors of urea. These can be divided into the *ureides*, restricting the term to those bodies which produce urea on hydrolysis, and the *non-ureides*, ammonia, carbamic acid, and cyanic acid, which produce urea by the synthetic processes of condensation and direct union.

1. Ureide precursors of urea: Reaction type:



Biochemical examples are guanidine, creatinine, arginine, allantoine, purine and pyrimidine derivatives, and alkaloids such as galegine. Of these, arginine is the only one definitely shown to produce urea by this process in the organism (285). The quantitative distribution of guanidine is so small that its biohydrolysis has not attracted much attention. Bell finds that parathyroid preparations have no appreciable effect on the decomposition of the free base, which is spontaneously hydrolysed at body temperature to ammonia and urea (26). Neither creatine nor allantoine give rise to urea by hydrolysis in the animal body (149), (5). The consideration of the purines and the pyrimidines is outside the scope of this review.

This difference in the stability of ureides so closely related as arginine and creatine suggests the existence of a specialised mechanism for dealing with the former. This has been experimentally proved by the discovery of the enzyme, arginase (165). Arginine is decomposed by arginase with the production of urea and the diamino-acid ornithine (45), (146), (147), (148):



The zymolysis is probably entirely localised in the organs that contain the enzyme, as arginase has not been found to occur in the circulating blood. Arginase is present in the livers of all animals with a *ureotelic* nitrogen metabolism, and is entirely absent from the livers of animals, such as birds, whose nitrogen metabolism is *uricotelic*. In mammals arginase is confined to the liver and kidneys; but is more widely distributed in sub-mammalian organisms. The zymolysis of arginine is never complete, 15 per cent to 30 per cent of the substrate remaining undecomposed (120). The reaction is inhibited by the presence of ornithine but not by urea, which suggests that the decomposition may not be as simple as the equation indicates. Ureide hydrolysis is not a very important source of urea in the animal body. It constitutes at the most 10 per cent of the total urea excreted on a mixed dietary (59), the exact amount depending on the quantity of arginine present.

2. The non-ureide precursors of urea. These comprise keto-cyanic acid, HN:CO, carbamic acid, $H_2N{\cdot}CO{\cdot}OH$, and ammonia. All have been claimed to occur in the circulating blood, but it is not possible to ascribe to any one the chief part in urea formation, although ammonia must participate ultimately in all the syntheses (249). Cyanic acid may arise directly from oxidative deamination or from the dehydration of carbamic acid, or, indirectly, from the oxidation of ammonia in presence of hydrocarbon nuclei. It does not appear to occur in the blood of the dog (233), (69), but is present some hours after a protein meal in the blood of man, the cat and the rabbit, in concentrations of 0.7 to 1.2 mgm. per 100 mls. (216), (69). It disappears from blood on keeping owing to hydrolysis. Carbamic acid may be formed either from the dehydration of ammonium carbonate or the hydrolysis of cyanic acid (70). The detection and estimation of carbamates in blood and in urine has evoked a considerable controversy (227), (228), (234), (3),

(4), (129), the fullest recent discussion of the subject will be found in the papers of Macleod and Haskins (193), (194) and in Werner's Monograph (299). The analytical methods employed do not distinguish finally between cyanates and carbamates, and as either acid may be produced from the other it is probable that when they occur at all in blood they are found together.

Ammonia may arise either directly by deamination or indirectly by the hydrolysis of cyanic acid and carbamic acid. The ammonia content of blood is much less than was formerly believed, and it is frequently absent or within the limits of experimental error (224), (236), (237), (189), (91). Much of it may arise from an external source, namely the putrefactive decompositions in the alimentary canal (85).

THE MECHANISM OF UREA FORMATION IN THE ANIMAL BODY. Here, two distinct processes are concerned: *a*, deamination, which sets free the urea precursors from the amino-acids; and *b*, synthesis, which converts the precursors into urea. The first process must be considered in detail, as its nature will determine the course of the subsequent synthesis.

Deamination. This is usually represented as a simple oxidation with formation of the ketonic acid or aldehyde corresponding to the original amino-acid, the amino-group being converted into ammonia:

$$R - CH_2 - CH(NH_2) \cdot COOH \xrightarrow{+O} R - CH_2 - CO - COOH + NH_3$$

This equation is based on the work of Neuberg, Dakin and others (232), (49), (272). Here, a general difficulty arises as to the formation of ammonia by oxidation in alkaline solution (299), (68), and on experimental grounds "it cannot be stated with certainty that ammonia is an obligatory product in the deamination in mammals" (81). Nor will the direct formation of ammonia explain the chemistry of the process. Lippich (180) and Dakin (49) have suggested alternate methods of deamination through the formation of uramido-acids, and Knoop (164) has considered the formation of hydrated amino-acids as the intermediate stage, but their production has not been experimentally demonstrated. To overcome these difficulties and to account for the appearance of cyanic acid when amino-acids are oxidised in alkaline or neutral solution, Fearon and Montgomery have considered an alternative mode of deamination in which the amino-group is removed as cyanic acid, thus providing a direct link between deamination and urea formation. In neutral solution the reaction may be written:

$$\begin{array}{l} R \cdot CH - - NH_3 \\ \quad | \qquad\quad | \\ \quad C \text{———} O \end{array} + O \rightarrow R \cdot CHO + HN : CO + H_2O$$

(*Amino-acid in cyclic form*)

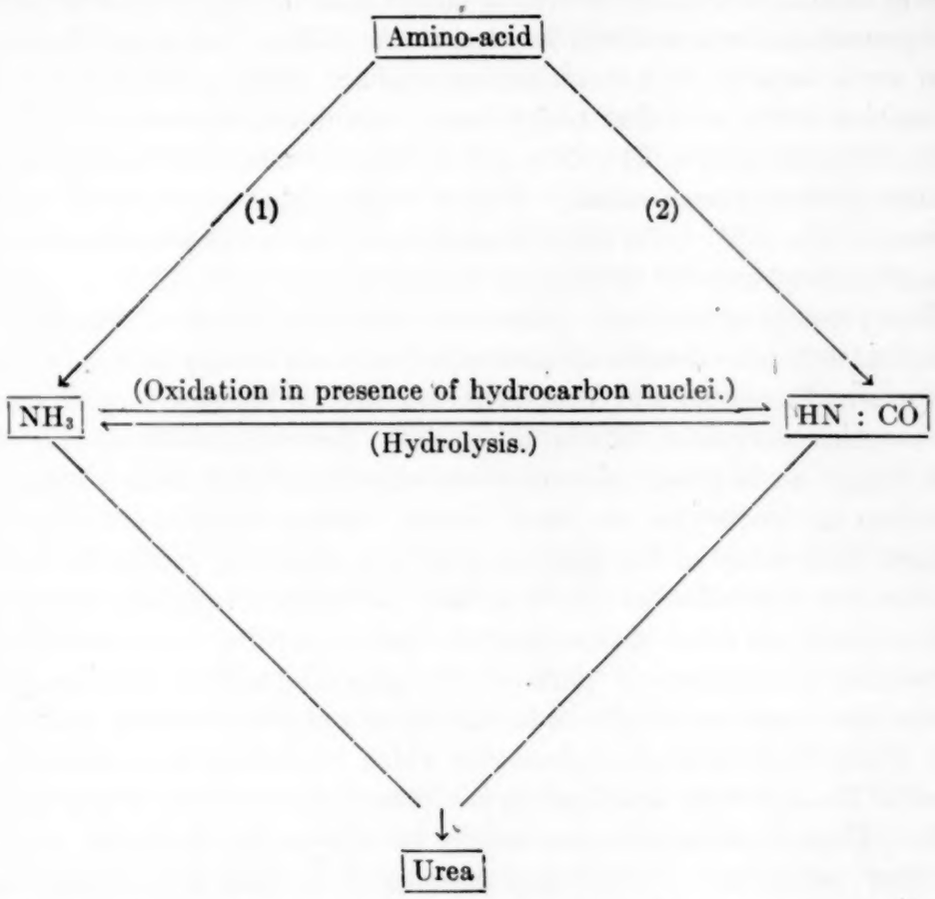
This view (68) is in agreement with the conclusions of Wieland and Bergel (302), who find that glycine, alanine, and phenyl-alanine when oxidised by means of activated charcoal yield an aldehyde with one carbon atom less than the original amino-acid, together with ammonia and carbon dioxide in equimolecular proportions. Hydrolysis of the cyanic acid would account for the production of these latter compounds.

A third form of deamination may be brought about by the spontaneous equilibrium formation of ammonia and the corresponding ketonic aldehyde when the amino-acid is incubated at body temperature in faintly acid solution:

$$R \cdot CH_2 \cdot CH(NH_2) - COOH \rightleftharpoons R \cdot CH_2 \cdot CO \cdot CHO + NH_3$$

This change, discovered by Dakin and Dudley (50), may be of considerable biological importance.

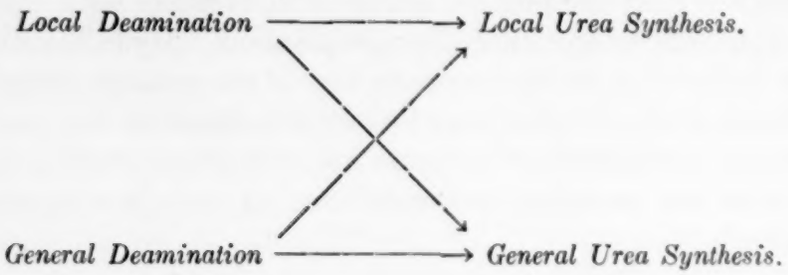
GRAPHIC SUMMARY OF THE METHODS OF UREA FORMATION FROM AMINO-ACIDS.



(1) Deamination by direct formation of ammonia, either through oxidation or reversion.

(2) Deamination by direct formation of cyanic acid.

The location of urea formation in the animal body. Each of the two processes concerned, deamination and synthesis, may be, in theory, general or local. Consequently, four possibilities must be considered:



Furthermore, the occurrence of deamination in a particular tissue or group of tissues does not imply that urea synthesis, if localised, is confined to the same tissues. The formation of urea in animal metabolism may be the result of two distinct and histologically separate events. When the early experiments of Schröder had shown the urea-forming power of the liver (268), it was assumed that deamination was part of the process and was entirely located there (153). Later and more general work showed that deamination was not always followed by urea formation (190), and that many lower organisms, bacteria (275), (33), (34), (31), and yeasts (64), were able to liberate ammonia from naturally occurring amino-compounds. This was also shown to occur in animal tissues (181), (152), (47), which strengthened the belief that deamination is a primitive property residing in all active cells (168), (32).

Experiments carried out under more rigid conditions of asepsis indicate that autolytic deamination does not occur as readily as was formerly believed. Hoagland and Mansfield obtained no increase in urea during the aseptic autolysis of muscle (139), (190). Levene and Meyer (173) find that amino-acids escape deamination when incubated with sterile suspensions of leucocytes or renal tissue. Observations such as these suggest that many of the positive results obtained by earlier investigators are due to accidental infection (32). Oxidation is closely associated with at least one form of deamination, and, according to Schweizer, the deaminase mechanism is part of the general oxidase system (272). Hence, the negative results obtained by recent workers may be due to lack of active oxygen, an explanation which is all the more likely on account of the fact that deamination is known to occur in the living kidney (224). Deamination must necessarily be a property of organs, such as the liver, which have been found capable of producing urea when perfused with amino-acids, but the absence of synthetic urea does not imply that there has been no deamination, although it strongly suggests it.

From the work of Dakin and Dudley (50), deamination to a slight

extent may be expected to occur throughout the organism owing to the spontaneous liberation of ammonia from amino-acids.[2] During the passage of amino-acids from the intestine to the blood deamination does not occur (86). Over 80 per cent of the absorbed amino-acids may escape immediate acceptance by the liver (290), (291), and thus become available for the general tissues, which take them up readily but in varying amounts. Glycine is more effectively utilised by muscle than by liver, alanine more by renal tissue than by muscle (184), (185).

The presence of deamination products in the blood. If deamination be a general process the products of deamination might reasonably be expected to occur in the circulating blood, either on their way to a local centre of urea synthesis, or as overflow products from the tissues. When the subject is examined a fundamental difference is found to obtain between animals of different species, which may explain some of the contradictory results arising from physiological investigations on urea formation. Blood drawn from certain animals such as man, the cat, and the rabbit, has a low ammonia content, which may be in the region of zero but which increases on standing to a figure three or four times the original value (208), (253), (132), (236), (237), (189). Dog's blood, on the contrary, does not show the presence of this labile ammonia-forming substance (224), (189). Now human blood, the blood of the cat and the rabbit, two to three hours after a protein meal, contain cyanic acid as an alkaline cyanate, the slow hydrolysis of which would entirely account for the gradual increase of ammonia in drawn blood. Dog's blood does not contain cyanic acid (within the experimental limits of the analytical method), and the ammonia content is not increased on standing.[3] This may imply that the dog differs from the other animals mentioned in that the processes of deamination and urea synthesis are not histologically separated to the same extent, but may be chiefly or entirely confined to the one organ.

It is known that the proteoclastic tissue enzymes of the dog are much more powerful than those of the rabbit (2), and, furthermore, the dog differs from man in the fact that the starvation output of urea even over long periods of time, may be very little below the normal value (145), thus indicating a more effective and economical mechanism for the production of urea.

Experimental methods employed in the study of urea formation: *1. Blood analysis.* Analysis of the afferent and efferent blood supply of various organs has been undertaken as long ago as 1859 (244), and been repeated

[2] Recent work of Bliss (29a) leads him to believe that ammonia formation is a general tissue phenomenon.

[3] The absence of cyanate from dog's blood has recently been confirmed by Gottlieb (118a).

frequently since then (122), (242), (160), (282). The results so far are not conclusive. This is probably because such variations as might reasonably be expected to occur in the urea concentration lie beyond the range of precision of the analytical methods which have been employed.

2. Tissue analysis. Urea is present in all the animal tissues examined, but the differences in concentration are not sufficient to determine a localised formation, nor if it were demonstrated that one particular tissue had a higher average urea content would this serve to distinguish between the function of urea formation and capacity for urea storage (122), (222), (144), (160), (267), (134), (84), (87), (89), (40), (202), (158), (280), (139), (138), (214), (114), (108).

3. Perfusion of organs. The method of hepatic perfusion introduced by Ludwig has been used to show the power of the liver to produce urea from amino-acids and the easily dissociated ammonium salts of organic acids (265), (268), (269), (261), (79), (154), (183), (80). Other organs have not been investigated to the same extent since the early work of Schröder (268), which showed that neither the kidneys nor the muscular tissue were able to produce urea from ammonium carbonate, within the limits of the analytical method employed.

4. Removal of organs. It has been proved by removal of the kidneys (247), (198), (268), (242), (209), (30) and the spleen (238), (286), (211) that these organs are not essential for the production of urea. Disconnection of the liver by the method of Eck's fistula, or by complete removal of the organ, is followed by a decreased production of urea in frogs (226) and in dogs (229), (276), (124), (57), (259), (240), (241), (197), (30). When at the same time the excretion of urine is terminated, by removal of the kidneys, it is found that the blood urea remains at a fixed value. Hence, the most recent workers, Bollman, Mann, and Magath, conclude that "production of urea in the dog is entirely dependent on the presence of the liver" (30), (198). This conclusion is not supported by the results obtained by previous workers on the frog (246), (119), the duck (262), the cat (81), and the dog (178), (206), most of whom agree in ascribing to the liver an active part in urea formation but deny that it is indispensable. Bollman, Mann and Magath seek an explanation for this diversity of opinion in the relative ineffectiveness of the methods employed. "The results of experiments when the liver was not entirely removed should not be interpreted as meaning complete suppression of the functions of that organ" (30).

Bollman, Mann and Magath have estimated the urea in the blood and urine of seventy-five hepatectomized dogs that lived from six to thirty-four and a half hours after removal of the liver. Whenever urinary se-

cretion was maintained there was a marked decrease in the urea content of the blood, this was accompanied by a decrease in the urea content of the tissues. Administration of amino-acids led to their increase in the blood but did not affect the decrease in the blood urea. These investigators have employed a greatly improved technique for removal of the liver (198), and their results may be regarded as conclusive. There remains, however, the possibility that urea formation is a general property of animal tissues but is completely overshadowed by the intense metabolic activity of the liver, and can only appear when the hepatic production of urea is reduced to a degree insufficient for the needs of the organism. This possibility must be considered on account of the work of Matthews and Miller (206), who succeeded in keeping one of their experimental animals, a dog, alive for fifteen months after an Eck's fistula had been established, and when "the liver has undergone degeneration almost to complete destruction." During the latter part of the dog's life its urea output rose steadily, and for two months maintained a value in the region of 80 per cent of the total nitrogen output. This indicates either that the liver residue was able to retain and to develop to an extraordinary degree the power of urea formation, or that the other tissues of the organism were able to assume this office (223).

However, under normal conditions it is unnecessary to invoke the aid of a reserve property of the tissues which is still hypothetical, the general accumulation of evidence in the case of the dog supports the conclusion of Bollman, Mann, and Magath. Acute yellow atrophy, atrophic and hypertrophic cirrhosis, and other conditions involving the destruction of hepatic tissue have been found associated with a diminished excretion of urea (223), (278), (110), (136). This association though frequent is not invariable, and no definite conclusion emerges. "The extent of the lesions and the functional capacity of the liver may vary considerably, and the influence on other tissues is always to be considered" (30).

5. Metabolic experiments. Folin and Denis (84), (85), (86) have found that urea, glycine, alanine, aspartic acid and asparagine are rapidly absorbed into the blood stream from the intestine in an unchanged form. The results definitely exclude both deamination and protein regeneration as features of amino-acid absorption. The absorbed amino-acids are taken up by the muscular tissue, the urea content of which subsequently increases. These results were interpreted as excluding definitely the doctrine that urea formation is localised in the liver.

Van Slyke and Meyer (290), (291), (292), have observed a similar accumulation of absorbed amino-acids in the liver; the saturation limit of which is 125 to 150 mgm. amino-nitrogen per 100 grams tissue as

compared with a limit of 80 mgm. amino-nitrogen per 100 grams muscle. The amino-nitrogen content of the liver decreases rapidly, and may be at the normal value within a period of three hours. The amino content of the other saturated tissues has hardly changed during the same interval of time. Decrease in the amino-nitrogen content of the liver is accompanied by an increase in the urea content. Consequently, Van Slyke concludes that the liver is the centre of both deamination and synthesis, and that it is continually tending to decrease the amino-acid content of the blood to the lower equilibrium limit, which in starvation is about 4 mgm. amino-nitrogen per 100 mls.

This conclusion presupposes an immediate and rapid formation of urea accompanying the rapid disappearance of absorbed amino-acids from the liver. Van Slyke has described experiments in support of this theory, showing that the urea content of the blood begins to rise directly the gastric contents enter the duodenum (292). Folin has recently criticised Van Slyke's work and conclusions, and has maintained his previous belief that urea formation is a general property of the tissues, especially the muscles, and that the greater mobility of the amino-acid content of the liver is due not to active deamination but to vascular transportation to supply the amino-acids of the blood stream, which are continually being consumed by the tissues.

Folin has found that in man the predominant increase in the non-protein nitrogen of the plasma during the early stages of absorption is associated mainly with an increase in the amino-acid nitrogen, and in the later stages with an increase in the urea nitrogen (89). That is to say, general distribution of the amino-acids throughout the organism precedes urea formation. In endeavoring to reconcile these results with the conclusions of Bollman and of Van Slyke it should be observed that these investigators have employed the dog as their experimental material, whereas Folin's recent work has been in the human subject. Morgulis (218a) working with dogs quite recently has shown that after a protein meal "the urea N of the systemic blood frequently increases *before* the amino acid N," and that "not infrequently a fall in the amino acid N of the blood has been found in the early stages of the digestive process." Two dogs, each with a true Eck fistula, showed a very low blood urea value of 2.9 to 6.4 mgm. per cent one month after the operation; the amino-acid nitrogen being normal, and the non-protein nitrogen only slightly below the value usually found in canine blood. While this additional evidence is in accordance with the criterion of Folin and Berglund (90), it is directly opposed to their conclusion in that it supports the belief that the liver is the predominant organ for urea formation. Here, again, the difference in conclusion may be due to the difference in the experimental material.

Conclusions as to the site of urea formation in the animal. On theoretical grounds, deamination is a process that may be expected to occur in any tissue capable of attaining the oxidation potential necessary for the liberation of the amino-group. Different amino-acids vary in the ease with which they undergo deamination, leucine being more resistant than either glycine or alanine (68). The selectivity manifested by the various tissues is probably determined by differences in the stability of the amino-acids. Where the products of deamination are cyanic acid and ammonia the subsequent process of urea formation may be expected to occur in any tissue since no further apparatus is required. Where the products of deamination are carbon dioxide and ammonia the subsequent formation of urea requires the operations of dehydration or oxidation; these are probably specialised and may well be located in one particular organ. The differences in the occurrence of labile precursors of ammonia in the blood of different species suggests a difference in the nature and location of the deamination process. *Experimental evidence from a variety of independent sources indicates that in the dog urea formation is located in and normally confined to the liver. Experimental evidence obtained from the human subject indicates that urea formation is not the unique property of any one particular tissue.*

THE FUNCTIONS OF UREA IN THE ANIMAL BODY. *A waste product.* The primary function of urea is to provide a stable, inert, and extremely soluble form in which nitrogen can be eliminated.

A buffer system. The precursors of urea form a buffer system capable of neutralising acids and ammonia. This is due to the properties of cyanic acid, which is very reactive in three ways of biological significance. It may undergo complete hydrolysis to produce ammonia and carbon dioxide, or it may undergo partial hydrolysis to produce urea, or it may unite directly with ammonia to form urea (299), (68).

$$+ H_2O + HR \rightarrow NH_3HR + CO_2 \qquad (1)$$

(*Acid hydrolysis*)

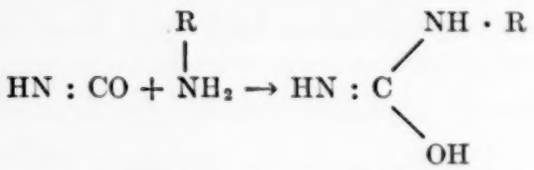
$$HN : CO \rightarrow + NH_3 \longrightarrow HN : C(NH_2)O \qquad (2)$$

$$+ H_2O + HN : CO \rightarrow HN : C(NH_2)O + CO_2 \qquad (3)$$

This unique property of cyanic acid in producing either a base or a neutral compound according to the conditions of hydrolysis suggests a more precise explanation of one of the factors for the maintenance of neutrality in the organism, and the diversion of nitrogen to the formation of ammonia in conditions of acidosis (128).

A diuretic. Urea has long been regarded as a diuretic, and was formerly employed for this purpose in therapeutics. Its effect is probably chiefly osmotic, but there also is evidence of a direct action on the renal tissue (24), (25).

A galactogogue. Addition of urea up to the effective maximum of 150 grams *per diem* to the diet of cows raised the milk production over a period of 100 days (293), (126), the increased yield in some animals being 6 to 8 per cent (143). This is probably associated with the diuretic property of urea and is not a specific effect, although Morimoto has observed a 50 per cent increase in the urea concentration of cow's milk immediately after calving (219). The changes recorded in the blood urea accompanying pregnancy and parturition are probably without effect on lactation (130). Lastly, there is the possibility long suspected but never demonstrated that urea formation is associated with the formation of other nitrogen-containing constituents of the urine. Uric acid, xanthine, and creatinine can all be regarded as urea derivatives, and ingenious suggestions have been put forward to account for their production either as by-products or extensions of the urea synthesis (274), (204). The reactions whereby methylamine and ethylamine are converted into substituted ureas may represent another form of biological detoxication:



(Amine) *(Substituted Urea)*

Urea exerts a slight myotonic action, especially on heart muscle, the extent of the effect being determined by the normal blood urea concentration of the animal. It is most marked in the elasmobranchs (196), (204). The effect resembles that of ammonium salts, and may be due to zymolysis of the urea owing to infection of the solution or the tissue. A retardation-acceleration effect has been observed to follow the addition of small quantities of urea to the lactose fermentation system (254), but the significance is unknown.

Urea as a protein sparer. In spite of the evidence for the irreversibility of urea formation in the animal body (294), (155), speculation has

been made as to the possibility of urea replacing, supplementing, or sparing protein in the dietary (218). Retention of administered urea may be partly explained by delayed excretion (264), (283), and partly by its conversion into ammonia in the alimentary tract (18), (6), (7). Any apparent effect of urea on protein consumption is almost certainly due to ammonia formation before it is absorbed into the blood stream, and the problem of the nutritive value of urea is only another way of opening the controversy as to the utilization of ammonium salts in nutrition (41).

The toxicity of urea. The fact that urea is a constant constituent of animal tissue, attaining to a very high value in some species of fish, indicates that it is not *toxic* in the ordinary sense of the term.

When administered in quantities sufficient to disturb the osmotic equilibrium of the organism urea exhibits, as might be expected, toxic properties. Thus, 100 grams of urea ingested has raised the blood urea to value of 160 to 245 mgm. per 100 mls. This has been accompanied by "headache, dizziness, fatigue, and other symptoms of asthenic uraemia" (137), (171). Here, the effect will be opposed by the ability of the kidneys to excrete urea.

Injection of urea in quantities of 1 per cent of the body weight is fatal to dogs. In lower concentrations it appears to have no influence, and is eventually excreted unchanged (135). The toxic value is determined by the fact that the animal is unable to excrete urea beyond a maximum rate, which in the dog is estimated to be 1.5 grams urea per 1 gram kidney tissue in 24 hours. Urea administered to rabbits in quantities of 1 to 2 grams per kilo of body weight produces severe toxic symptoms and death (24), (25). The possible effects of ammonia formed from urea in the alimentary tract must be taken into account in discussing the effects of urea administration (18). This will be specially important in animals which possess a gastric urease (188). Fish can live for days without apparent ill-effects in urea solutions up to 2 per cent (142).

Uremia. Urea was formerly regarded as the compound responsible for the whole series of pathological conditions termed *uremia*. Now, it is only associated with the azotemic or nitrogen-retention group (35), (191); and even here the principal toxin is probably not urea. Beaumont and Dodds (20) record in one year four typical cases with blood urea values below 40 mgm. per 100 mls. However, the opinion seems well founded that even "if urea is not responsible for uremia, it is a good index of the severity of the condition" (296).

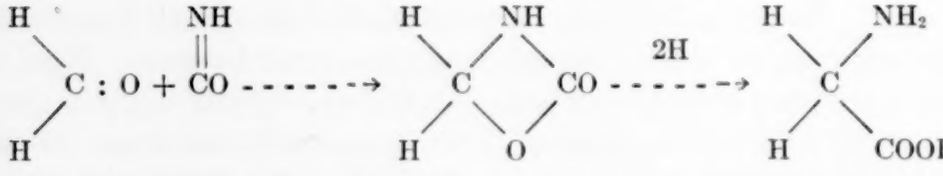
THE FORMATION OF UREA IN PLANTS. Among green plants, Prjanschnikoff (248) has shown that excess of ammonia is converted into as-

paragine, which he regards as being formed in the plant in the same way and for the same purpose as urea in the animal. Asparagine, furthermore, is the chief decomposition product of protein metabolism in bean leaves, and appears to be the principal mobile form of nitrogen in the green plant (40). It differs essentially from urea in not being excreted but in being stored up and possibly utilised later to form protein. As Werner has pointed out (299), it is very probable that the appearance of urea in the metabolism of higher plants is the result of a side reaction and not one of the main transformations. The plant is probably able to utilise the end-products of its own endogenous protein metabolism for the synthesis of fresh protein without the necessity of passing the end-products through the soil. How, then, does urea enter into the biochemistry of the higher plant? Werner, following the work of Fosse (99), considers that urea is a constant product of the breaking down of vegetable protein during the germination of seeds, before the plantule has developed the power of synthesising carbohydrates. The urea is later on decomposed by the enzyme urease, which, according to Morimoto (220), is an enzyme of great importance in plant protein synthesis. Urea supplied to the soil is readily decomposed by the soil organisms and also by the root-nodules of the *Leguminosae*, which are rich in urease (300). Urease dissociates urea into ammonia and cyanic acid (66), either of which may participate in protein synthesis. Werner considers that cyanic acid formation represents the first stage in the synthesis of the plant proteins:

$$CO_2 + NH_3 \rightarrow HN : CO + H_2O$$

This transformation has recently been accomplished by the ultra-violet irradiation of solutions of carbon dioxide and ammonia (71).

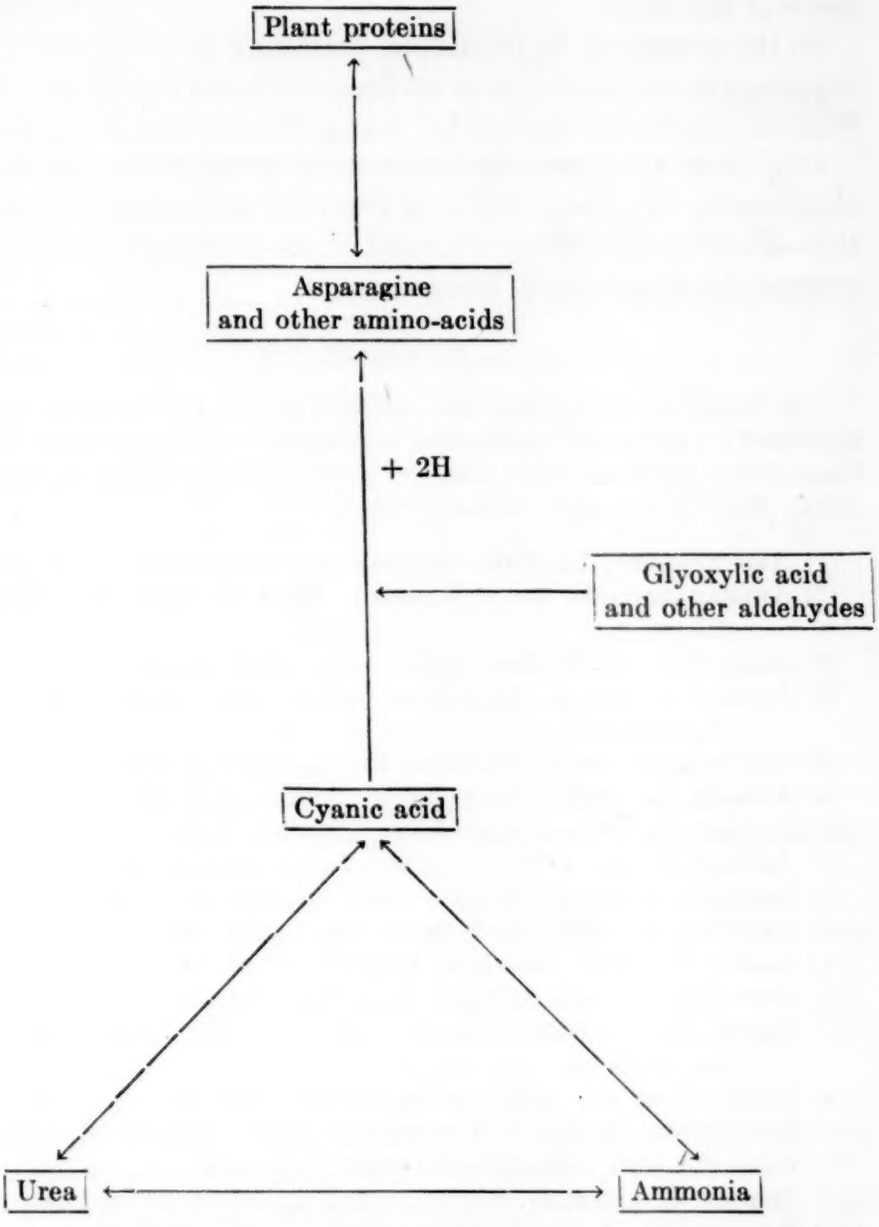
Cyanic acid once formed might unite with an aldehyde, and by a reduction process the converse of that suggested to explain the cyanic type of deamination the corresponding amino-acid might be produced:



The non-chlorophyll containing plants are much richer in urea than the green plants (95), (154). The accumulation of urea is associated with a carbohydrate deficiency (155), and may represent a diversion of nitrogen from protein formation, or, as is less likely, a deamination in excess of the immediate nitrogen requirements.

The detection of cyanuric acid, the polymer of cyanic acid, (306) indicates the occurrence of active zymolysis of urea in the soil.

GRAPHIC SUMMARY OF THE NITROGEN CYCLE IN PLANTS. The following hypothetical summary has been compiled to incorporate the views of Fosse and Werner on the importance of cyanic acid in plant metabolism.



The glyoxylic acid, and other aldehydes, are presupposed to arise during the carbohydrate synthesis. In the absence of sufficient carbohydrate the nitrogen is diverted to the formation of urea. In the absence of sufficient nitrogen the glyoxylic acid is diverted to the formation of oxalic acid.

THE BIOCHEMICAL SIGNIFICANCE OF UREA. Urea is among the most soluble and least toxic of the organic nitrogen compounds. Within the animal body it is a stable end-product, unlikely to experience any oxidative or reductive changes. Outside the animal body it is readily decomposed by the widely-distributed urealytic enzymes, and the nitrogen, liberated in a highly reactive form, is able to enter into the life-cycle of the plant.

In the process of its formation, urea may be of service to the animal organism in various ways; in its decomposition it may provide the plant with an important reactant for the synthesis of amino-acids.

Only from one aspect is urea a waste product; it also represents the chief carrier of organic nitrogen from the animal to the plant, and in this affords an additional example of the economic soundness of those commercial transactions known as life.

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THE SECRETION OF URINE

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The main channels for the elimination from the body of the waste products formed in metabolism as well as various foreign substances which gain access to the blood are the kidneys, lungs, intestinal epithelium and skin. Elimination through the skin removes mainly water and serves more as a part of the heat regulating mechanism than as a mode of excretion. Carbon dioxide is the chief waste product eliminated by way of the lungs, but any volatile substance which has entered the blood stream will pass through the lungs according to definite physical laws of diffusion. Calcium, magnesium, phosphates, heavy metals and certain pigments pass out from the organism in greater or less amount by way of the intestine. With the above mentioned exceptions the kidneys are the organs upon which falls the task of the elimination of waste products. In fact, the kidneys excrete to some extent practically all substances which occur in a diffusible form in the plasma, while non-diffusible bodies or colloids are normally not eliminated by these organs.

It is frequently stated that the function of the kidney is to eliminate waste products from the body. Such a statement does not, however, do justice to these important organs. The ability of the kidney to prevent the elimination of useful substances such as glucose, and to excrete chlorides and water in large amounts when present in excess but to markedly decrease their elimination when the body lacks them, is not taken account of in such a definition. The function of the kidney can probably be better stated to be to keep the blood plasma more or less constant in composition. The elimination of waste products, such as urea, naturally is included in such a definition for since they are continually being produced in the body they must be eliminated to prevent their concentration in the blood rising above normal. Changes in the composition of the plasma are reflected in the urine. If there is an excess of sodium chloride or urea, an increased rate of elimination of these bodies results; if there is a deficiency of chloride in the plasma, a decrease or cessation in the elimination of this substance occurs. When

the hydrogen ion concentration of the blood rises above normal, a more acid urine is excreted; if it falls below normal, a more alkaline urine is eliminated. On the other hand, when marked lowering of the bicarbonate concentration in the plasma is produced, e.g., by the ingestion of ammonium chloride, little chloride is eliminated, although the chloride concentration in the plasma is considerably above normal. The sum of chloride and bicarbonate is, however, normal. This simply means that where the composition of the plasma cannot be kept normal as regards all of its constituents, there is an effort on the part of the organism to maintain a constant osmotic pressure rather than a constant concentration of any one constituent.

In the present review, the secretion of the urine will be discussed from two main points of view. Since the first elucidation of the anatomical structure of the kidney, attempts have been made to formulate theories explaining the secretion of urine, and to try and assign functions to the different parts of the kidney. On the other hand, many investigators have not concerned themselves with the intimate details of the mechanism of renal excretion but have looked upon the kidney as an organ transforming blood plasma into urine, and have tried to find out how changes in the plasma or in the conditions in the general organism will alter the urine. In other words, they have attempted to find the stimulus to which the kidney responds by a change in urine quantity or quality. This latter method of approach involves more or less the important question of the regulation of urinary secretion, and is particularly valuable as it can be applied to the normal intact animal or to the human subject. As more is learned about the mechanism of urinary secretion these two methods of approach will become more mutually dependent. At present they supplement each other.

Owing to the limitation of space in this review, many important phases of the physiology of the kidney will have to be omitted or at least only touched upon as they concern the general topics discussed. The blood flow and metabolism of the kidney, the action of diuretics and other drugs, the production of albuminuria, hemoglobinuria, and glycosuria, as well as other important subjects cannot be adequately dealt with.

THEORIES OF URINARY SECRETION. The theories which have been proposed to explain the mechanism of renal excretion date from the classical paper of Bowman in 1842 in which the true relations of the glomerulus and tubule were pointed out and the unit of kidney structure identified. As is well known, Bowman in this paper proposed his theory

of urinary secretion. This, however, was based entirely on structural grounds and it was only in 1874, when Heidenhain began the publication of his studies on the kidney, that experimental data which seemed to support the theory became available. The so-called Bowman-Heidenhain theory was the result of this work. In the interval, and in fact a few years after Bowman's publication, Carl Ludwig published his theory of urinary secretion. Compromises between the two theories have naturally been suggested by many writers.

The publication of the monograph on the secretion of the urine by Cushny in 1917 marks a distinct advance in the attempts to formulate a satisfactory theory of kidney activity. His presentation of the mass of data which had accumulated and his suggestion that all of the apparently contradictory facts could be fitted into a simple theory of urinary secretion, a theory stated in such clear cut and definite terms that it could easily be subjected to experimental testing, has aided greatly in clarifying the subject and has, moreover, stimulated a great deal of new investigation in this field. The theory which Cushny designates as the "modern theory" may be stated briefly as follows. The glomeruli filter from the blood all of the diffusible constituents of the plasma. This glomerular filtrate contains, therefore, practically all the non-colloidal constituents of the plasma in nearly the same concentrations as they exist in the blood. During the passage of this fluid along the tubules, water and various constituents (threshold bodies) are reabsorbed, while other substances (non-threshold bodies) are gradually concentrated during their course along the tubule. The reabsorbed fluid has, moreover, an unvarying composition, approximately that of Locke's solution. In its original form this theory is, however, no longer tenable and must be modified at least to the extent of postulating some reabsorption of threshold bodies and a fluid of quite variable composition.

The problem which concerns us in the present state of our knowledge in connection with the mechanism of urinary excretion is to determine the function of the various parts of the renal unit—the glomerulus and different parts of the tubule. Moreover, one must try to decide if the process by which water and dissolved substances pass through the glomerulus is a process of filtration so clearly suggested by its histological structure, or if it is some more complicated physico-chemical process not at present clearly recognized. What is the function of the tubule in general and of its different parts in particular? Does a transfer of water and dissolved bodies take place from lumen of tubule to lymph and blood (reabsorption) or is the transfer in the other direction from blood to

lumen (secretion)? If we retain the terminology given by the earlier writers, the processes which have been extensively discussed in connection with the renal mechanism are filtration, reabsorption, and secretion. A brief review will now be attempted of the evidence which exists for these processes taking place in the kidney.

Function of the glomerulus. In discussing the function of the glomerulus, the evidence that it is the chief site of fluid elimination in the kidney will be first considered. In Bowman's classical paper this idea was suggested from purely structural considerations. Nussbaum in 1878 carried out his famous work on the amphibian kidney. He found that due to the double blood supply of the kidney in amphibia, ligature of the renal arteries abolished the blood supply to the glomeruli, but the tubules still received blood via the renal portal vein. When this procedure was successfully performed on frogs, the elimination of urine ceased. These observations, confirmed by many (Dreser, 1885; Schmidt, 1891; Halsey, 1902; Beddard, 1902; Bainbridge and Beddard, 1906), and the structural arguments first used by Bowman have convinced practically all investigators that the glomerulus is the main, if not the entire route, for fluid elimination from the blood. All theories of urinary secretion include this as their starting point. The long drawn out controversy between the adherents of the Ludwig and the Bowman-Heidenhain theories gives evidence, however, that there was little agreement as to the nature of the process by which fluid passes through. Glomerular filtration and glomerular secretion represented the two opposing views. At the present time most workers accept filtration as the best working hypothesis of glomerular function. A few, however, still seem to adhere to secretion as the process involved in the glomerulus, while Woodland (1922, 1924) even denies the participation of the glomerulus in urine formation, considering it as a pressure regulator, somewhat according to the ideas of Lamy and Mayer (1906) and Brodie (1914). We will now consider the evidence upon which the theory of filtration is based.

Since the investigations of Goll (1854) in Ludwig's laboratory, it has been known that changes in general blood pressure result in corresponding changes in the rate of urine flow, and this has been urged as an argument in favor of glomerular filtration. Experiments have been performed showing that changes in renal blood pressure produced either by stimulation or section of the vasomotor nerves to the kidney or by partial occlusion of the renal artery produce changes in urine flow in the same sense as the changes in blood pressure (Bernard, 1859; Hermann, 1862; Ustimowitsch, 1870). These have been confirmed

by many subsequent workers. All of these experiments, however, involve not only changes in renal blood pressure but also changes in the volume flow of blood through the kidney. This latter factor was urged by Heidenhain as the important one. The experiments of Richards and Plant (1922), in which the kidney was successfully perfused with a mechanical pump so that the volume flow of blood could be kept constant but the pressure varied, seem to demonstrate in an unequivocal manner that changes in renal blood pressure, without concomitant changes in the velocity or volume flow of blood through the kidney, produce corresponding changes in the rate of urinary elimination. Bainbridge and Evans (1914) have utilized the heart-lung preparation made from one animal, to perfuse the isolated kidney of another. Starling and Verney (1922) have recently developed and utilized this preparation very extensively. Although both the pressure and volume flow of blood to the kidney can be accurately determined at any time, alterations in pressure may be accompanied by changes in volume flow. However, in a number of instances, they have observed changes in the rate of urine flow accompanying changes in pressure where the blood flow through the organ was unchanged (Dreyer and Verney, 1923). Thus, it can be concluded that a change in blood pressure, without an accompanying change in volume flow of blood, will produce a change in a similar direction in the rate of urine flow. It is difficult to reconcile this fact with the idea of glomerular secretion of fluid, but it is readily interpreted and demanded by the theory of glomerular filtration.

The fluid eliminated by the glomerulus can be regarded as normally free of protein (Posner, 1880; Seelig, 1891; Wearn and Richards, 1924; White and Schmitt, 1925–1926), although the idea that this fluid may contain protein which disappears during its passage through the tubule has been maintained (de Haan, 1922; de Haan and Bakker, 1923). The other constituents of the blood plasma, at least the diffusible ones, must be present in the glomerular fluid in approximately the same concentrations as in the plasma, if filtration occurs, because the renal blood pressure is not sufficient to overcome the osmotic pressure of any of the diffusible constituents.[1] If the proteins are retained by the filter,

[1] This statement must be qualified to some extent. Since the osmotic pressure of the plasma proteins has never been determined by a satisfactory method, and since the glomerular capillary pressure is more or less a matter of guess work, it is quite possible that the glomerulus might be impermeable to some of the constituents occurring in the plasma in small amounts (sulphates, phosphates, etc.) and filtration still take place. This would be still more possible if any of the

the blood pressure (or filtration pressure) must always be slightly greater than the osmotic pressure of the retained colloids if any filtration is to occur. When the blood pressure falls to the level of the osmotic pressure of the colloids, filtration should cease. The osmotic pressure of the colloids of blood plasma has been found to be about 20 to 30 mm. of mercury, (Starling, 1899; Schade and Clausen, 1924; Fahr and Swanson, 1926), while the elimination of urine ceases in an animal with normal plasma when the general blood pressure falls to about 35–40 mm. of mercury. If the colloids of plasma are reduced in concentration by dilution of the blood, formation of urine will occur at a much lower blood pressure than before. It follows from the above argument that the difference between the maximum pressure in the ureter, against which urine can be eliminated, and the glomerular blood pressure should be equal to the colloid osmotic pressure of the plasma. The ureter pressure has been found to depend on and vary with the blood pressure (Starling, 1899; Henderson, 1905).

Other experiments which seem to argue strongly in favor of the glomerular function being filtration, were carried out by Barcroft and Straub (1910–11) (compare Tamura and Miwa, 1920) on the oxygen consumption of the kidney and its relation to diuresis. They found that an enormous increase in the rate of urinary flow, caused by the injection of Ringer's solution into a cat, was not accompanied by any increase in the oxygen usage of the kidney. This would seem to argue for a mechanical explanation of the increased flow of urine.

Many attempts have been made to eliminate the action of the tubules and so obtain a pure glomerular fluid. Bainbridge, Menzies and Collins (1913) showed that when the frog's kidney was perfused both by the renal arterial and renal portal system with oxygenated Ringer's solution, at the pressures which normally obtained, a hypotonic fluid escaped from the ureter. On the addition of a trace of mercuric chloride to the renal portal perfusion fluid, the fluid issuing from the ureter became isotonic. They believed that none of the poison reached the glomeruli, which

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plasma bodies exist in a more complex form with a higher molecular weight. Such may be the situation in the case of phosphates. The idea of Hamburger and his co-workers (Hamburger and Brinkman, 1918; Brinkman, 1919; Hamburger, 1923) that the glomerular membrane is impermeable to glucose and other sugars would appear to be untenable on the filtration theory. This idea, however, has been disproven by the work of Clark (1922), Wearn and Richards (1924), and White (1925). The possibility of another explanation of his results is even admitted by Hamburger.

were producing a filtrate. Barcroft and Straub, by repeated bleeding and replacement of the blood by Ringer's solution, obtained from a cat a urine which contained the same concentrations of urea and chloride as the plasma. They attributed the results to asphyxia of the tubules. Starling and Verney (1925) have succeeded by perfusion of the kidney with blood containing small amounts of cyanide to inhibit the part played by oxidative processes in the formation of the urine, in obtaining a urine which was practically identical with the plasma in composition.

Wearn and Richards (1924) punctured Bowman's capsule in the living frog, by means of a fine capillary, and obtained protein-free glomerular fluid. Qualitative tests have shown that the glomerular fluid may contain both chloride and glucose, when the bladder urine collected at the same time is free of both substances. Although this, of course, is direct evidence for reabsorption of these bodies, the presence of glucose and chloride in the glomerular fluid, when the urine contains none, would seem to point to glomerular filtration rather than secretion. White and Schmitt (1925, 1926) have confirmed these results for the kidney of Necturus. Wearn and Richards (1925) have extended their investigations along this line to a quantitative comparison of the chloride concentration in glomerular fluid and in blood plasma. The chloride concentration of the glomerular urine was estimated by the nephelometric method of T. W. Richards, and a comparison made of the figures with the plasma content of chloride. Their results and conclusions are stated as follows. "In ten of thirteen experiments the plasma Cl concentration was also determined. In all but one of these, glomerular Cl was higher than plasma chloride. In seven, the difference amounted to more than 15 per cent of the latter; in four, more than 28 per cent. Conceding the possibility of errors of technique amounting to 10 per cent, we are forced to conclude that the filtration theory, as at present defined, is not adequate to explain our results. If subsequent work shall confirm these, it is clear that the processes concerned in glomerular filtration require further study." An extension of work along this line should prove especially valuable and instructive.

Many arguments have naturally, at one time or another, been directed against the filtration theory. A good presentation of these may be found in the writings of Heidenhain (1883) and of Brodie (1914). To their proponents at the time, some of these arguments seemed unanswerable; but, as new facts have been brought to light and our understanding of the situation has become clearer, satisfactory answers have been

found to most of these. We can discuss here only two or three of the most important of these objections. The fact that partial occlusion of the renal vein of an animal, which must raise the pressure in the glomeruli, leads to a diminution instead of an increase in the rate of urine formation was one of Heidenhain's strongest arguments against filtration. Tammann (1896) was the first to point out that the slowing of the blood stream, caused by this procedure, might effect such a concentration of blood and hence of colloids in the glomerulus, so that the effective filtration pressure was not increased. Richards and Plant (1922) have shown that this is probably the true explanation of the discrepancy, since they found that when the volume flow of blood through the kidney was kept constant, partial occlusion of the renal vein resulted in the expected increased rate of urine formation. The opposite experiment of clamping the renal artery was also used as evidence against filtration. It was generally believed that when after a few minutes' closure the artery is unclamped, the elimination of urine is not restored until a long time (as much as an hour or more) after the return of the circulation. It seemed impossible to assume that a mechanical filter could be completely deprived of its function by such treatment. The argument has never seemed of great weight because the glomerulus, if a filter, is a filter composed of living cells which might easily be seriously injured by a short period of anemia. It has been recently found that the facts upon which the argument was based are incorrect; that after clamping the renal artery for a few minutes (or over 20 minutes) the secretion of urine starts almost immediately after reëstablishing the circulation (Marshall and Crane, 1923). This statement has been somewhat discredited by Stoll and Carlson (1923), but confirmed by Livingston (1925). It seems that when a temporary anuria is brought about by this procedure, it is due to a diminution of blood flow through the kidney and not to the previous short period of anemia.

Another argument that has been given considerable prominence by the opponents of filtration, is that the elimination of the amount of urea normally found in urine would entail the filtration of an impossible quantity of fluid. An example may make this clearer—human blood plasma contains about 300 milligrams of urea per litre, while the kidneys of a man may excrete from such a plasma 1500 cc. of urine, containing 2 per cent of urea, per day. If urea is eliminated entirely by filtration, as suggested by Cushny's modern theory, this would mean that 100 litres of plasma would have to be filtered and 98.5 litres reabsorbed each day. The fact that this amount of urea can be eliminated

from a plasma of such a low concentration is established: the kidneys extract the urea from 100 litres of plasma, if not by filtration, by some other means. Cushny has given an interesting calculation of the amount of filtration that each glomerulus of a cat's kidney must form under similar conditions. We will modify the calculation for the human kidney. The two human kidneys contain somewhere in the neighborhood of 9,000,000 glomeruli (Traut, 1923), each with its corresponding tubule. In the example we have given above, this would mean that each glomerulus must filter only 0.0000077 cc. and each tubule reabsorb about 0.0000076 cc. per minute, not an impossible task by any means. Of course, this objection, such as it is, does not apply to a combined theory of filtration and secretion, admitting the secretion of such substances as urea.

Before leaving the discussion of the glomerulus it is necessary to refer to the investigations of Richards and Schmidt (1924) which have given us a new conception of glomerular filtration. These investigators have found, by using methods of direct microscopic observations of the living frog's kidney, that the conditions in the glomerular circulation of the kidney are similar to those described by Krogh for skeletal muscle. Only a certain proportion of the glomeruli may be functioning at one time. A glomerulus may exhibit an intermittance in the flow of blood through its capillary tuft and, indeed, only in some and not in all capillaries of the same glomerulus may a flow of blood be seen. Various vaso-constrictor agents may markedly diminish the number of active glomeruli, while vasodilator or diuretic substances may render active all the glomeruli of a kidney. By the injection of various substances into the living animal and histological examination of the kidney, Khanolkar (1922) and Hayman and Starr (1925) have shown that this idea applies to the mammalian kidney. Hayman and Starr found in the anesthetized rabbit that 56 to 89 per cent of the glomeruli were functioning; by injection of adrenalin this number could be markedly reduced, while the administration of diuretics caused all the glomeruli to become active. The fact that all units of the kidney are not always functioning, introduces the conception into renal physiology that the extent of the glomerular capillary (filtering) surface may undergo marked variation.

Functions of the tubules. The part played by the tubules in the elaboration of urine will next be discussed. The structure of the tubule is so markedly different from that of the glomerulus, that all theories of urinary secretion have assigned to it a different function. The histo-

logical structure of the different segments of the renal tubule also suggests that these histological differences must have some functional significance. However, since we do not as yet know definitely nor do we find any agreement among investigators concerning the functions to be assigned to the tubule it is impossible to consider the different segments of the tubule separately.[2]

The evidence that reabsorption of certain substances takes place in the tubule is just as satisfactory and of a much more direct nature than that for glomerular filtration. The reabsorption of certain substances (glucose, chloride, etc.) follows as a necessary consequence of filtration, since these substances are always present in blood plasma, but may be completely absent from the urine. In addition, since chlorides may be present in the urine of mammals in a much higher concentration than in the plasma, the reabsorption of a certain amount of water must be postulated unless one assumes secretion as well as reabsorption of chloride. In view of the direct evidence for reabsorption which has been obtained in the amphibian kidney, the various lines of indirect evidence present in the literature will not be discussed in much detail.

Cushny (1901–02, 1902, 1904) states that after the injection of a mixture of sodium chloride and sodium sulphate into the blood in such amounts that an equal number of anions is present, the amounts eliminated in the urine follow different curves, and the percentages in the urine are independent of the concentrations in the plasma. When the urine flow from one kidney is decreased by partial obstruction of the ureter, chloride is present on the obstructed side in both lesser absolute amount and percentage, while sulphate appears in a smaller absolute amount but higher percentage. Diminishing the urine elimination from one kidney by partial occlusion of the renal artery leads to similar results (Yagi and Kuroda, 1915). The explanation of these results is found in a more ready reabsorption of the chloride than of the sulphate of the glomerular filtrate in its passage along the tubule. Phosphate and urea, when injected, behave in a manner similar to sulphate, while bicarbonate was found to resemble chloride. An interpretation of these results can be given upon the basis of secretion by the tubule; and Brodie and Cullis (1906), who found slight ureteral pressure to increase the urinary flow as well as the quantities of sulphate and chloride elimi-

[2] In the paper by White and Schmitt (1926) which has just appeared, direct evidence is presented for the reabsorption of sugar and chloride in the proximal convoluted tubule of the kidney of Necturus.

nated, favor this view. These latter results are attributed by Cushny (1917) to some dilator reflex from the ureter.

Nishi (1910) examined the cortex and medulla of the kidney for sugar and found that while the cortex contained from 0.011 to 0.066 per cent, sugar was either absent from the medulla or present only in traces. When glycosuria was produced both the cortex and medulla contained sugar. These results appear to indicate a reabsorption of sugar by the tubules under normal conditions. The experiments of Clark (1922) on perfusion of the frog's kidney both through the aorta and the renal portal vein are even more conclusive of the reabsorption of sugar. The appearance and amount of glucose in the urine was found to depend on the glucose concentration of the fluid bathing the tubules and not upon the sugar content of the arterial perfusate.

Other evidence for reabsorption by the tubules appears to be less satisfactory and less direct than that already mentioned. (Ribbert, 1883; Boyd, 1902; Sobieranski, 1895; Bainbridge, Collins and Menzies, 1913; Bainbridge, Menzies and Collins, 1914.)

Direct evidence of reabsorption in the frog's kidney has been given by Wearn and Richards (1924). They succeeded in obtaining fluid from a single glomerulus of the frog's kidney and found that it might contain both glucose and chloride, while the bladder urine collected during the same period showed no trace of these substances. This has been recently confirmed by White and Schmitt (1925, 1926) on Necturus.

The evidence is sufficient to allow most investigators in this field to accept filtration and reabsorption. The important point of discussion at present is what substances, if any, are secreted by the tubules. Heidenhain's original proof of the secretion of a foreign dyestuff, indigo-carmine, by the tubules after this substance is injected into the blood has been criticised by many investigators (Cushny, 1917), and since it has now been shown by direct methods that this substance is present in the glomerular fluid of the frog (Wearn and Richards, 1924), the experiment is no longer an entirely convincing one. The numerous investigations which have been carried out with dyes following the famous indigo-carmine experiment have not given definite results (for a review see Turchini, 1922). One difficulty is to decide whether the dye has entered the cells from the lumen or the blood. The possibility that these dyes have entered the tubular cells from the lumen during fixation and were not present there during life was never satisfactorily eliminated by the earlier investigations. Hayman (1925) has recently shown that this can happen in the frog. He injected certain dyes directly into a

glomerular capsule, in another series of experiments intravenously and found the kidney after fixation to show the same histological picture. Ghiron (1913) studied the living kidney of a mouse under the microscope, and followed the changes produced by the injection of dyes. He states that the dye first appears in the lumen and later in the cells, due to reabsorption. Neither Khaolkar (1922) nor Edwards (1926) were able to confirm his observations. However, Edwards and Marshall (1924) and Edwards (1926) have found by direct microscopic observation of the kidney of the living frog and rat that certain dyes can be seen in the cells of the proximal convoluted tubules after intravenous injection.

Dawson (1924) has utilized the peculiar arrangement of tubules in Necturus to study tubular function. Two types of renal tubules are present in the mesonephros of Necturus (Chase, 1923): tubules connected with the body cavity by peritoneal canals entering the glomerular neck; and other tubules, more dorsal in position, which lack peritoneal canals. Iron salts (intraperitoneal injection) which reach the lumina of the tubules from the body cavity and not via the blood stream, can be found, after fixation by the Prussian blue reaction, in the epithelium of the convoluted tubules, while iron salts which reach the kidney by the blood stream can be located in the glomerulus. The objections to this type of experiment would appear to be the possibility of diffusion of iron into the cells during fixation and the possible toxic effects of the large amount of iron which must be given. Edwards, in unpublished work from this laboratory, has shown by direct observation of the living kidney of this animal that certain dyes (tetra-chlor phenol red, phenol red, etc.) are not reabsorbed from the lumen of the ventral tubules.

Admitting the presence of certain dyes in the cells after an intravenous injection and proof that they did not reach the cell by reabsorption, their presence does not conclusively prove their secretion. Some of the more recent investigators of renal function by the use of dyes argue in favor of filtration and reabsorption alone being able to explain their results (von Mollendorf, 1922; Mitamura, 1924). This phase of the subject has been confused by the fact that many writers working with different chemical substances have drawn conclusions concerning the elimination of dyes in general. It seems certain that each dye or at least groups of dyes, must be studied and considered separately (Rohde, 1920; Schulten, 1925; Edwards, 1926; Grollman, 1926).

A similar line of work has been carried out with the use of various micro-chemical staining reactions to show that both foreign substances

and substances normally present in the plasma are eliminated through the epithelium of the convoluted tubules. As an example we may consider the widely quoted evidence for secretion of urea obtained by the histo-chemical method. Leschke (1915) claims to have demonstrated localized concentration of urea (as well as uric acid, chloride and phosphate) in the epithelium of the convoluted tubules. Oliver (1916) has confirmed his observation for urea, but admits that the reaction used is not specific for the purpose, and Walter (1923) is still more doubtful of its value. Policard (1915) using the more specific xanthydrol reagent of Fosse (1914) could find no precipitate of urea in any tubule cells; but Chevallier and Chabanier (1915), with the same method, detected urea in the cells of the convoluted tubules, all blood vessles of the kidney, and the lumina of the ducts of Bellini. This method has been subsequently employed by Oliver (1921), Stubel (1921), Piras (1922), Walter (1923) and Hollman (1923). Their findings, while differing from one another in some minor details, seem to confirm Chevallier and Chabanier in localizing the crystals of dixanthyl urea in the epithelium of the convoluted tubules, the lumina of all tubules including the glomerular capsule, the tissue spaces between the tubules, and the lumina of the blood vessels. Using the liver as a control, Oliver and Piras find the precipitate only in the blood vessels and not in the hepatic cells, while Walter states that it is to be seen in both. All of these observers conclude that their data prove the secretion of urea by the cells of the convoluted tubules, while Walter adds that his results disprove the filtration theory of the glomerulus as this structure must also actively secrete urea. It seems to us obvious, however, that the conditions under which the precipitation of urea with xanthydrol occurs must be more carefully studied before any conclusions can be drawn from this type of experiment. Oliver assumes that there is a threshold in cellular protoplasm below which no precipitate is formed, but that such a threshold (or at least as high a one) does not exist for blood or partially elaborated urine. The best results were obtained when the urea concentration in the urine was increased by protein feeding or injection of urea. Possible explanations of the results without assuming a localized concentration of urea in the cells of the convoluted tubules would be the existence of substances inhibiting the precipitation in the renal cells, other than those of the convoluted tubules and the cells of the liver, or that the urea has diffused post mortem from its more concentrated solution in the lumen into the cells. It is difficult, however, on the latter assumption to see why the cells of the medullary tubules

should not also show the presence of urea. The determination of the urea content of the whole kidney (Cushny, 1917; Mayrs, 1923) gives no support to the idea of a concentration of urea in any cells of the tubules, while Marshall and Crane (1924) have found by chemical methods that urea is present in a lesser concentration in the cortex than in the medulla of the mammalian kidney, and that the urea content of the cortex is about the same or somewhat less than that of the plasma. Addis (1925) has confirmed these observations. A repetition of one of Oliver's experiments, determining the urea content of cortex and medulla chemically instead of relying on histochemical reactions, has shown that urea is not concentrated in the convoluted tubules as would be indicated by the xanthydrol reaction (Marshall and Crane, 1924).

The secretion of many other substances both normal and foreign to the organism has been claimed as the result of histochemical investigations (Glaevecke, 1883; Kobert, 1883; Ebstein and Nicolaier, 1896; Minkowski, 1898; Sauer, 1899; Anten, 1901; Biberfeld, 1904, 1907; Courmont and André, 1905; Roehl, 1905; Basler, 1906; Leschke, 1915; Stieglitz, 1921; Firket, 1921). These observations are no more satisfactory as evidence than the xanthydrol results which are certainly misleading.

The classical experiment of Nussbaum (1878) on the frog's kidney, utilizing the double blood supply and indicating a secretion of urine by the tubules when urea is injected intravenously, is a matter of textbook knowledge. Certain objections to this experiment and difficulties in its interpretation have been pointed out by Cushny (1917). By perfusion of both the renal arterial and renal portal systems of the frog, at appropriate pressures, Atkinson, Clark and Menzies (1920) found that the tubule cells transfer urea from the perfusion fluid to the urine, but the relative concentrations in perfusion fluid and urine were not determined. Sulphate was also transferred, but glucose was not. The objection that simple diffusion might account for the results cannot be answered until the relative concentrations of the substances in perfusion fluid and urine are determined. If it could be shown that urea and sulphate are more concentrated in the urine than in the perfusion fluid and that these substances actually do not reach the glomeruli, this experiment would constitute direct evidence for secretion of these substances by the tubules of the frog's kidney. Trevan (1916) had previously reported, in abstract, that perfusion of the renal arterial system with Ringer's solution gave a urine of the same reaction as the Ringer, but simultaneous perfusion of the renal portal system with neu-

tral phosphate gave an acid urine. Yoshida (1924) has recently used the method of double perfusion in a study of the elimination of a great many substances by the frog's kidney, determining quantitatively the percentages of the bodies in the urine. The lack of data for the composition of the fluid actually in contact with the tubules (easily obtained by an analysis of the fluid leaving the renal vein) when different solutions are used in the two perfusion systems, renders an interpretation of his results difficult. He finds that when sulphate is contained only in the renal portal fluid, the urine contains much less than this fluid, but that iodide and thiocyanate under the same condition are about as concentrated in the urine as in the renal portal fluid. This last observation may possibly show that these two substances are more concentrated in the urine than in the fluid actually bathing the tubules, and hence furnish evidence of their secretion, if they really do not reach the glomerulus.

Two other sets of facts which are frequently urged as evidence for secretion are 1, the finding of various kinds of granules and vacuoles in the cells of the convoluted tubules which are believed to indicate secretion, and 2, attempts to determine the site of elimination of various substances by producing a localized injury in the kidney by means of poisons. The paper of Gurwitsch (1902) may be consulted for the first of these, but it is evidence of the most indirect kind. In regard to the selective action of renal poisons, it is only necessary to point out that until proof can be given that their action is confined entirely to one part of the kidney, no certain conclusion can be drawn from the data. It is not necessary to have a recognizable histological change produced to have a functional alteration. Any attempt to separate the glomeruli and tubules in the mammalian kidney by blocking the glomerular vessels with particulate matter or oil (Lindemann, 1901) is certain of failure, because, even if successful, the injection would also cut off the blood supply from the tubules. The careful investigations of Huber (1907), Gerard (1911), Gross (1917) and Traut (1923) have proven that all of the blood supply of the tubules is derived from the efferent vessels of the glomeruli.

A number of investigators have presented indirect evidence which they have interpreted as pointing to secretion. Results have been obtained which are not in accordance with Cushny's "modern theory" of filtration and reabsorption, but they can possibly be interpreted by some modification of this theory without necessitating secretion. As examples of this type of evidence we may mention the studies of White

(1923) of the plasma and urine concentrations as well as of the rates of elimination of urea, sulphate, phosphate and glucose; the similar studies of Underhill (1923) on urea, uric acid, creatinine, and phosphate in the cat. The explanation of these results involves a selective and varying reabsorption of practically all urinary constituents, unless secretion is postulated.

In recent years more satisfactory evidence that the secretion of certain bodies occurs in the tubules has been presented. Marshall and Vickers (1923), studying the elimination of phenol red (phenolsulphonephthalein) by the kidney, concluded that this dye was eliminated by the convoluted tubules of the dog's kidney as well as through the glomeruli. Their evidence may be stated briefly. If the elimination of urine in a dog is prevented by the lowered blood pressure incident on section and partial destruction of the spinal cord and an intravenous injection of phenol red given, this substance is found in large amounts in the kidneys and in a very much higher concentration than it is present in the blood serum or other tissues. The dye is confined almost entirely to the cortex, and probably to the cells of the convoluted tubules. They noted, further, that phenol red is present in the plasma partly in a non-diffusible combination with the proteins, so that only a part of the total amount in the blood is available for filtration through the glomerulus. [The nature of this binding to the colloids as well as the adequacy of the methods adopted to measure it has been examined by Grollman in two recent investigations (1925, 1926).] Under certain conditions it was found that the amount of filterable phenol red in the blood plasma reaching the kidneys was less than that which was eliminated in the urine in a certain time, so that its excretion cannot be explained on filtration alone *even if all the plasma were assumed to be filtered.* After injection, phenol red is apparently stored in large amounts in the cells of the convoluted tubules and then gradually secreted into the lumen of the tubules. The maximum rate of elimination of this body does not occur immediately after injection when the plasma concentration is at its height, as would be expected if it were entirely filtered, but some time later. When a mixture of urea and phenol red is injected into the blood of a dog in increasing amounts and the rate of excretion of these two bodies compared to their plasma concentrations, quite different relations are found. The rate of excretion of urea is found to be proportional to its plasma concentration, while this is only true for phenol red at low plasma levels, as its rate of excretion becomes nearly constant at higher concentrations (Marshall and Crane, 1924).

Starling and Verney (1925), making use of the heart-lung-kidney preparation, have confirmed the accumulation of phenol red in the tubule cells, as well as the binding of the dye by the serum colloids, and have also found that when the kidney is perfused with cyanided blood phenol red appears in the filtrate in less concentration than it exists in the blood-plasma while all of the normal urinary constituents examined appear in the same concentration. They also conclude that phenol red is secreted after a preliminary concentration in the tubule cells.

Results obtained by de Haan (1922) on the relative concentration of certain dyes and urea by the rabbit's kidney would indicate either that certain dyes (including phenol red) are secreted by the tubules or that the glomerular filtrate normally contains the colloids of the plasma which disappear during its passage along the tubules. Even on the basis of this latter alternative, which is eliminated by direct evidence (Wearn and Richards, 1924) one has to assume a considerable reabsorption of urea. Eliminating the possibility of the glomerular fluid containing protein, and assuming that the concentration of substances in this fluid is approximately the same as in blood plasma, de Haan's results would necessitate the reabsorption of over 99.5 per cent of the urea and of practically the same amount of all other normal urinary constituents, if secretion of dye does not occur. Although there is probably some error in the methods used by de Haan for determining the amount of dye bound to the colloids (Grollman, 1925, 1926) the above figures are certainly of the correct order of magnitude. In the dog, whose plasma binds much less phenol red than the rabbit's, experiments in this laboratory have indicated that even during an active diuresis one must assume a reabsorption of over 90 per cent of urea unless secretion of phenol red occurs.

An argument based on the relative concentration ratio (concentration of a substance in the urine divided by the concentration of that substance in the plasma) has been developed by Mayrs (1924) for the secretion of uric acid by the bird's kidney. This substance is concentrated by the fowl's kidney to a much greater extent than any of the other bodies normally occurring in the urine. Mayrs interprets this as meaning that uric acid is secreted by the bird's kidney, and unless one accepts this idea, a reabsorption of a very large amount of the other urinary constituents must be postulated. He believes that secretion is a more primitive process than filtration and accepts the secretion of dyes by the mammalian kidney as a survival of a process occurring in a more primitive kidney. Marshall and Crane (1924) find that urea is concentrated to a

much greater extent than other substances occurring in frog's urine; and that, in addition, urea may occur in the frog's kidney in a much greater concentration than in the blood or other tissues. They eliminated the possibility of the increased urea content of the kidney being due to the contained urine, and localized the concentration in the dorsal portion of the kidney which contains mainly proximal convolutions (the ones with brush border). Phenol red can also be shown chemically to be concentrated in the dorsal part of the frog's kidney. The conclusion was drawn that both phenol red and urea are secreted by the convoluted tubules of the frog's kidney. Bieter and Hirshfelder (1924) from a microscopic examination of the kidney of living frogs after the injection of phenol red or indigo carmine, concluded that these dyes are eliminated entirely by the glomeruli and concentrated in the lumen of the tubules by reabsorption of water. This conclusion is in opposition to the results of Marshall and Vickers (1923) on the dog's kidney and of Marshall and Crane (1924) on the frog's kidney, but is possibly explained by the microscopic observations of Edwards and Marshall (1924). Bieter and Hirshfelder could find no dye in the cells of the tubules, but they observed only the ventral surface of the kidney, where glomeruli and distal convolutions can be seen, and not proximal convolutions. It is on the dorsal surface, where proximal convolutions occur, that concentration of the dye in the cells can be seen. Later, Hirshfelder and Bieter (1925) admit that phenol red can be seen in the cells on the dorsal surface, but do not believe that it is more concentrated there than in other parts of the organism.

The work of Nash and Benedict (1921), which seemed to show conclusively that the ammonia of the urine was formed in the kidney probably from urea or amino-acids, would, if correct, necessitate the secretion of ammonia by certain cells of the renal tubules. In brief, they found the renal vein blood of the dog to contain two to three times the concentration of ammonia found in the arterial blood, while blood of the inferior vena cava below the kidneys contained about the same quantity as arterial blood. In addition, bilateral-nephrectomy or ureteral ligation did not cause any accumulation of ammonia in the blood. The formation of ammonia by the kidney has found general confirmation and acceptance in the publications on the subject that have appeared since Nash and Benedict's paper (Hendrix and Bodansky, 1924; Loeb, Atchley and Benedict, 1924, Russell, 1923; Rabinowitch, 1923, 1924; Ambard and Schmid, 1922). Bliss (1926) has recently reinvestigated the question of the site of ammonia formation, and although his experi-

mental findings are in close agreement with those of Nash and Benedict, his conclusion is that ammonia formation is a generalized tissue phenomenon. The failure of the blood ammonia to increase after bilateral nephrectomy is explained by the elimination of large amounts of ammonia in the vomitus, and blood ammonia values are believed to be increased in nephritis. Although it is still possible from these results that a great part of the ammonia formed by the kidney finds its way directly into the urine from the cells in which it is formed, Bliss believes that ammonia finds its way into the urine like other nitrogenous waste products from the arterial blood. Even on the basis of this idea, it is still difficult to reconcile the elimination of ammonia by the kidney with a process of filtration alone. Since the ammonia content of arterial blood in man is normally not over 0.10 mgm. per cent, ammonia must be concentrated to a very great degree by the kidney, so much so that one has to assume on the filtration theory alone an extensive reabsorption of the other urinary constituents. This would seem to be less likely than the secretion of ammonia by the tubules.

Hippuric acid, the synthesis of which from benzoic acid and glycocoll was proven by Bunge and Schmiedeberg to take place in the kidney of the dog, is probably secreted directly into the urine from the cells in which it is formed. Subsequent work has cast some doubt upon the kidney being the only site of synthesis (Kingsbury and Bell, 1915; Kingsbury and Swanson, 1921) although Snapper, Grunbaum and Neuberg (1922, 1924) maintain that in the dog the kidney alone is concerned, while in other species, other organs may participate.

The only other substances which are concentrated to any great extent by the kidney are creatinine,[3] sulphates, phosphates and under certain conditions glucose. These, although not concentrated to exactly the same extent as demanded by the "modern theory" (Mayrs, 1922; White, 1923; Underhill, 1923) have concentration ratios of the same order of magnitude. Marshall and Crane (1923) used temporary asphyxia of the kidney by occlusion of the renal artery in an attempt to distinguish between substances which might be excreted by the kidney entirely by mechanical filtration and those which might be eliminated partly by secretion. They showed that occlusion of the renal artery for 20 minutes gave rise to no change or to an increase in the water, chloride and bicarbonate eliminated after the occlusion, whereas there was a

[3] The work of Behre and Benedict (1922) has led to some doubt of the existence of creatinine in the blood. Until more information is available, it would seem best not to discuss creatinine results.

distinct decrease in the amounts of urea, phosphate, sulphate, creatinine and ammonia excreted. This evidence, however, is indirect, and a possible explanation can be given in terms of filtration and reabsorption alone for these bodies.

Starling and Verney (1925) obtained results similar to those noted above as the result of asphyxia on the isolated kidney, and also by the use of cyanide to abolish oxidative processes. In addition, they compared the urine eliminated by the isolated kidney when perfused alternately with the heart-lung preparation and normal blood, and with a pump and blood containing hydrocyanic acid. The mechanical conditions and the chemical composition of the blood were identical in the two cases, except that blood in the pump circuit contained a trace of cyanide. The results showed a marked fall in the absolute amounts of urea and sulphate when the preparation was switched from the heart-lung to the pump circuit, followed by an increase during the recovery on returning to the heart-lung circuit. In addition, the percentages of urea and sulphate were nearly the same as those in the plasma during the perfusion with cyanided blood. Since the blood pressure and blood flow through the kidney was not appreciably different in one circuit than in the other, the authors conclude that the amount of glomerular filtrate must be the same in the two cases, and hence that urea and sulphate are actively secreted into the glomerular filtrate by the cells of the tubules. However, they found no evidence of a preliminary concentration of urea and sulphate in the kidney, as has been shown for phenol red. Therefore, although phenol red, urea and sulphate are all secreted, the mechanism of the secretion of urea and sulphate is quite different from that of phenol red. If it could be proven beyond question that poisoning the kidney with cyanide does not change the rate of glomerular filtration, these experiments would certainly prove the secretion of urea and sulphate. The authors, however, have assumed that cyanide affects only the functions of the tubule, and not those of the glomerulus. But since the glomerulus is composed of living cells, the oxidative processes of which are abolished by cyanide as well as those of the tubular cells, the amount of glomerular filtrate with the same mechanical conditions may be markedly diminished in the cyanided kidney. In fact, Eichholtz and Starling (1925) in a subsequent communication admit that cyanide is not without influence on the permeability of the glomerulus. They believe, however, that cyanide increases the permeability because protein begins to appear in the urine after about ten minutes perfusion with cyanide and because phosphates, to

which they hold the glomerulus is not normally permeable, pass into the fluid from the cyanided kidney. Even admitting this increased permeability to protein and phosphate, it does not necessarily follow that the rate of filtration of fluid is increased; it may be diminished. It has been shown that with a collodion filter one may increase the permeability so that substances of large molecular volume like proteins or colloidal dyes appear in the ultrafiltrate, but the rate of filtration (of fluid) may be markedly diminished by the same procedure (Grollman, 1926). It is possible to prepare a collodion filter which allows a trace of protein to pass when a blood albumin solution containing bile salts is filtered, but which is not permeable to protein and is more permeable to water when the same solution and pressure are used without bile salts.

If the belief of Starling and his co-workers that the phosphate in normal serum exists as a "non-ionised colloidal calcium compound" to which the glomerular membrane is impermeable be true, secretion of phosphate by the tubule cells must be postulated. The evidence of White and Schmitt (1926) that phosphate is absent or if present exists in a much lower concentration than the plasma in the glomerular fluid of Necturus was obtained by the most direct means. Confirmation and extension of their results must be awaited together with data on the state of the inorganic phosphate in the plasma of this animal. Bock and Iversen (1921) find that the rate of excretion of phosphate is unchanged in water diuresis, but is markedly increased during theophyllin diuresis and is accompanied by a drop in the plasma concentration. White (1925) has demonstrated for the dog and Havard and Reay (1926) for man that phosphate may be much less concentrated in the urine than in the plasma. These results are explainable by assuming that an active reabsorption of phosphate from the glomerular fluid takes place under certain conditions, that the mammalian glomerulus filters only a portion of the phosphate present in plasma, or that secretion of phosphate occurs. The evidence at present available is in no way sufficient to allow one to conclusively decide between these possibilities.

Results obtained by ultrafiltration of mammalian serum show the complete filterability of the inorganic phosphate (Cushny, 1919; Neuhausen and Pincus, 1923). Recent results obtained in this laboratory (Grollman) prove that the addition of small amounts of calcium (insufficient to cause any precipitation of phosphate) to serum diminishes the amount of filterable phosphorus. This brings up the question of the condition of the phosphate in normal circulating blood, that is, in blood in which no changes have been produced by clotting, defibrination, or

addition of anticoagulants. When this has been determined, it may explain the fact that injected phosphates are readily excreted under conditions where the phosphate normally occurring in plasma scarcely appears in the urine. It is interesting to recall in this connection that many years ago Loewi (1902) suggested that both phosphate and sugar circulate in the blood in colloidal combinations, and that they can only be eliminated by active secretion of the tubule cells. Eichholtz, Robinson and Brull (1925) offer the suggestion that a considerable part, if not all, of the urinary phosphate results from the hydrolysis of organic phosphates in the kidney.

The question of the secretion of water by the tubules naturally arises for consideration; for, although evidence has been given that the glomerulus is the main site of fluid elimination in the kidney, the idea of some fluid being eliminated by the tubules cannot be disproven at present. It is possible either that substances which are secreted are added to the filtrate in the lumen of the tubule by some process not involving the transfer of water or that a true secretion takes place as in the true secretory glands, *i.e.*, the formation of a hypotonic solution of plasma constituents. The elimination of fluid by frogs, with ligated renal arteries, after the injection of urea was held by Nussbaum to prove that the tubules secrete urea in solution when stimulated by an excess of this substance in the blood. On the other hand, in the frog's kidney Bieter and Hirschfelder found that phenol red was not present in the lumen of the ventral tubules in the lower pole, the arterial blood supply of which had been interrupted, under conditions when the upper pole, with arterial supply intact, exhibited many tubules containing the dye. This result, which is in striking contrast to the experiments of Nussbaum who found that frogs with ligated renal arteries still excreted indigo carmine, would seem to show that water is no longer eliminated when the glomerular circulation is abolished. It does not, however, disprove the secretion or passage of the dye through the dorsal tubules of the frog's kidney without the concomitant excretion of fluid. A repetition of the "Nussbaum experiment" with careful analyses of blood and bladder fluid as well as more certainty of the lack of collateral circulation in the glomeruli would appear desirable.

We may summarize the evidence concerning the functions of the tubules as follows. Satisfactory direct evidence of reabsorption of chloride and glucose exists for the amphibian kidney, and a great mass of indirect evidence points to reabsorption of water and certain substances in the mammalian kidney. Evidence, which it is difficult to explain

otherwise and which has not been successfully criticized, also exists for the secretion of certain dyes (phenol red, etc.) and ammonia by the mammalian kidney, of uric acid by the bird's kidney, and of urea and phenol red by the frog's kidney. Other substances (urea, sulphates and phosphates[4]) are stated to be also eliminated partly by secretion by the mammalian kidney, but without preliminary concentration in the renal cells, by a mechanism differing from that of the secretion of phenol red. The evidence, however, for the secretion of these bodies is not entirely satisfactory at present, and involves certain implications which it is difficult to accept.

Summary of theories of urinary secretion. The passage of the non-colloid constituents of the blood plasma through the glomeruli by filtration, and the reabsorption of certain substances by the tubules would appear to be generally accepted by most workers. The "modern theory" as outlined by Cushny is insufficient to explain recent data on concentration ratios, and must be at least modified to include some reabsorption of practically all substances and a fluid of quite varying composition. The point discussed most actively at present is whether secretion occurs, if so what substances are secreted, and what is the relative importance of the glomeruli and the tubules in the elimination of these substances. Secretion is not accepted by some, who believe that filtration and selective reabsorption explain the whole process of urine formation. Secretion is assigned a prominent rôle by others; urea, phosphate, sulphate and other bodies being excreted largely by this process. Again, secretion is accepted, but to a limited extent in the mammalian kidney, where it is looked upon as a relic of a process occurring in a more primitive kidney, as suggested by Mayrs. It is seen that the question most actively discussed involves a knowledge of the amount of glomerular filtrate. Is this filtrate sufficient in quantity to account for all of the substances eliminated in the urine, or only for all of the urea and sulphate and bodies concentrated to a smaller degree than these, or is it of such limited quantity that part of the urea and sulphate must be secreted? It is well to point out that selective reabsorption is just as complicated a process as secretion, one being simply the reverse of the other.

To the reviewer, the following statement of the mechanism of the

[4] The question of the secretion of phosphate, owing to the possibility of part or all of the inorganic phosphate in the plasma being in a non-filterable form has been dealt with in detail. I am not aware of any evidence as to whether or not phosphate is concentrated in the renal cells.

secretion of urine appears in the light of all known facts to be the best working hypothesis for the mammalian kidney. All of the non-colloid constituents of the plasma are eliminated by filtration through the glomerulus; water, chloride, bicarbonate, potassium (possibly phosphate, uric acid and other bodies) are reabsorbed during the passage of the filtrate along the tubules; urea and sulphates are also reabsorbed to some extent under certain conditions but not as actively as chloride; ammonia, hippuric acid and possibly other bodies are formed in the renal cells and secreted, while certain foreign substances when present in the organism (phenol red) and possibly substances occurring in small amounts in the organism are secreted after a preliminary concentration in the tubule cells. The amount of glomerular filtrate on this hypothesis is considered sufficient to account for all of the sulphate and urea eliminated but not sufficient to account for ammonia, certain dyes (phenol red, etc.) and other bodies which are concentrated to a much greater extent than sulphate by the kidney.

Regulation of urinary secretion. If the mechanism by which urine is eliminated in the kidney were completely understood, one could easily deduce just how the regulation of this secretion as regards quantity and composition is brought about under different physiological conditions. From what is already known, however, certain deductions can be made and these are fortunately supplemented by the work of many investigators, who have been concerned more with the nature of the changes in the organism which cause certain definite responses on the part of the kidney than with the analysis of the function of the different parts of the organ. It appears at present that two main factors regulate the quantity and composition of the urine. These are changes in the glomerular blood pressure (and filtering surface) and changes in the chemical composition of the blood plasma.

Changes in the glomerular blood pressure in the kidney in man or in the intact animal may be brought about by changes in the general arterial blood pressure without a change in the caliber of the renal vessels, by changes in the caliber of the renal vessels without a change in the general blood pressure, and possibly by a greater constriction of the efferent than the afferent glomerular vessels as recently postulated by Richards (Richards and Plant, 1922; Mendenhall and Richards, 1924) to explain the diuresis in certain animal experiments. Variations in the number of glomeruli in the kidneys receiving blood as well as variations in the active capillaries of a single glomerulus will markedly change the filtering surface, and hence influence the amount of glomerular filtrate with-

out a necessary change in renal blood pressure. This would, of course, change both the quantity and composition of urine obtained from the bladder, but we possess at present no evidence as to how important these changes are. One can group changes in renal blood pressure, glomerular filtering bed, and tonus of the efferent glomerular vessels together under the circulatory control. Of course, circulatory changes may be due in the first instance to changes in the chemical composition of the blood.

We have very little evidence as to how important the circulatory method of regulation is under the ordinary conditions of life. The diminished excretion of urine which takes place during muscular exercise, even if water has been previously ingested, is explained as due to renal vasoconstriction by MacKeith, Pembrey, Spurrell, Warner and Westlake (1923–24). The diuresis resulting from baths (Bazett, 1924) has been ascribed to a change in renal blood pressure due to the hydrostatic pressure exerted on the abdomen (Griffiths and Hansell, 1925). It is also possible that emotional diuresis and the marked decrease of urine elimination normally occurring during sleep are due to circulatory changes.

Under ordinary conditions of life, the second method of control of urinary secretion, while not at all completely understood, appears to be of more importance than the circulatory one. It seems that any change in the chemical composition of the blood plasma will cause a change in the quantity or composition of the urine. Two general principles or laws may be used under which to discuss the data which have accumulated on this subject. These may be stated provisionally as follows:

1. The rate of elimination of any substance in the urine by the kidney depends on the concentration of this body in the plasma, if other influencing factors are constant.

2. A change in the concentration of one constituent of the plasma generally produces definite effects on the rate of elimination of other substances besides the one which has been varied.

Ambard and his school (Ambard, 1920) have been mainly responsible for directing attention to the first of these laws. This investigator divides the substances eliminated by the kidney into two classes, threshold (e.g. sodium chloride) and non-threshold (e.g., urea) bodies. The latter of these would continue to be eliminated in the urine as long as any of the substance remained in the blood plasma, while the former would cease to be eliminated when the concentration in the plasma had fallen to a certain limiting value, the threshold. Cushny has used the

terms, as stated above, to mean a substance which is or is not reabsorbed by the tubules, supposing this to be the explanation of their different behavior. For any non-threshold body, Ambard believed that its rate of elimination in the urine varied directly as the square of its concentration in the blood, and inversely as the square root of its concentration in the urine. Combining these two laws, he proposed the following equation governing the excretion of urea,

$$\frac{[\text{Concentration in blood}]^2}{\text{Rate of excretion} \sqrt{\text{concentration in urine}}} = \text{constant}$$

and the following modification for a threshold substance, sodium chloride:

$$\frac{[\text{Concentration of excess over threshold}]^2}{\text{Rate of excretion} \sqrt{\text{concentration in urine}}} = \text{constant}$$

These laws were supposed to hold true for the same individual or for individuals of the same body weight. Assuming that the rate of excretion varies directly as the weight of the individual, this factor of size could be introduced, rendering the law applicable to different individuals. In this form Ambard's coefficient, or some modification of it, has been used as a test of the renal function, and a large number of clinical papers have appeared concerning its value. We cannot discuss these in the present connection, but since Ambard's work was the first serious attempt to formulate definite laws governing the rate of elimination by the kidney, a further discussion of how far such laws or modifications can be applied must be undertaken. Although Ambard's two fundamental laws have not been confirmed by later investigators of the subject, he has undoubtedly stimulated much valuable research along these lines.

MacLean and Selling (1914) and MacLean (1915) tested Ambard's laws using much more accurate methods of analysis than did Ambard. Their general conclusion was that "the excretion of urea and sodium chloride in the normal individual is carried out according to definite laws, capable of numerical expression." Marshall and Davis (1915), studying the effect of the injection of large doses of urea, found that, when plenty of water is given, "the rate of excretion of urea in normal animals is directly proportional to the concentration of urea in the blood." Additional data indicating that there is a general tendency of the rate of urea excretion to vary directly with the blood urea concen-

tration have been published by Pepper and Austin (1915), Addis and Watanabe (1917), Addis, Barnett and Shevky (1918), and Austin, Stillman and Van Slyke (1921). Addis and his co-workers believe that no relation exists between the urea concentration or volume of the urine and the rate of excretion, while Austin, Stillman and Van Slyke concluded that the rate of excretion varies directly as the blood urea concentration and inversely as the square root of the urinary volume, provided this volume is less than a certain maximum. Addis and his collaborators, after many years' study of the excretion of urea, conclude that "there are many other factors than blood urea concentration which may influence the rate of urea excretion." The circulatory regulation mentioned above (a change in glomerular pressure or number of active glomeruli) is one of these, and changes in other plasma constituents is another, e.g., presence of adrenalin or pituitrin (Addis, Shevky and Bevier, 1918). These workers have finally been able to find conditions under which the ratio of the rate of urea excretion in the urine to the blood urea concentration becomes quite constant, that is to say, that the rate of excretion varies in the same subject directly as the blood urea concentration. These conditions are as follows. "At an early hour in the morning, usually about 6 a.m., the subject drank about 1,000 cc. of water in which a variable amount of urea had been dissolved. No food was taken. Every hour thereafter about 500 cc. of water were drunk. No measurements were made until at least 3 hours after the administration of urea. The urine was collected at hourly intervals and blood drawn at the middle of each period of urine collection." In the two subjects most carefully studied, the ratios of milligrams of urea excreted per hour to milligrams of urea per 100 cc. of blood were 46.1 with a variability of 6.85 per cent and 56.2 with a variability of 4.76 per cent. This was true over a change of about 20 to 100 milligrams per cent in the blood urea. Drury (1923) has found that under special conditions in rabbits the ratio remains constant over a change of blood urea concentration from 20 to 700 mgm. per cent.

In view of the remarkable constancy of the "ratio" which has been found under special conditions for the human subject, Addis and Drury (1923) have studied the influence of other factors than blood urea concentration on the rate of urea excretion. They found the rate increased by the administration of milk, caffeine and glutamic acid, and decreased by muscular exercise, pituitrin and large amounts of adrenalin. While the ratio for the subject Drury is 56.2 under the "standard conditions," it decreases to 46.5 when the ingestion of urea is omitted and still further

to 39.9 when neither water nor urea are taken, but is practically normal (57.8) when 1 per cent NaCl solution is substituted for the water and urea. Under the last two sets of conditions the urine volumes are small, while under the first two they are large. From these and other data, the authors conclude that the volume of urine has no demonstrable effect on the rate of urea excretion (Addis and Drury, 1923).

This relation between the rate of urea excretion and the urea concentration in the blood is exactly what would be expected on the basis of the theory of urinary secretion which we have adopted. The greater variations under conditions other than those finally adopted by Addis and Drury might be due to differences in the glomerular filtration rate (caused possibly by varying numbers of active glomeruli) and to differences in the reabsorption of urea by the tubules. The ingestion of large amounts of either urea or sodium chloride would appear to stabilize the rate of filtration, possibly by rendering all glomeruli active, and decreasing absorption of urea in the tubules. If no reabsorption of urea takes place under the "standard conditions," the Addis-Drury ratio would represent the amount of glomerular filtrate in 100 cc. per hour. It is, of course, equally possible to explain their results on the basis of the partial elimination of urea by secretion.

Sufficient information does not exist in regard to other substances to discuss in any detail the relationship of the rate of excretion to the plasma concentration. A beginning towards this in the case of phosphate has been made by Haldane, Wigglesworth and Woodrow (1924), Wigglesworth and Woodrow (1923–24), and Addis, Meyers and Bayer (1925). Their results indicate in a general way that changes in the rate of excretion are roughly proportional to changes in the blood phosphate concentration, although considerable evidence is available for the existence of a threshold for phosphates. Adolph (1925) from a study of the data of several investigators concludes that the excretory ratios for urea and phosphate are about the same as the Addis-Drury ratio, under other circumstances than the "standard conditions," if he assumes an apparent threshold for these substances. Under the standard conditions of Addis and Drury the threshold for urea disappears.

The marked increase in the rate of elimination of urine which occurs after the ingestion of large quantities of water by mouth has been the subject of much investigation. There is no reason to believe that the diuresis is due to changed circulatory conditions in the kidney, so that the stimulus for the increased urine flow has been sought in a change in the composition of the blood. The investigations of Engel and Scharl

(1906), Macallum and Benson (1909), and Haldane and Priestley (1916) have shown that there is no dilution of the blood in relation to its colloid, red cell or hemoglobin content. Priestley (1916), however, detected a slight fall in the electrical conductivity of the serum and concluded that water absorbed from the intestine did not remain in the blood stream, but was distributed in the tissues, and that salts passed out of the blood into the water in the alimentary canal or into the tissues to a marked extent. The work of Molitor and Pick (1922) would indicate the importance of the liver in this connection. Later, it was found that the water content of the blood was slightly increased during water diuresis (Priestley, 1921). It appears, therefore, that there is an increase in the water content of the blood, and a dilution of the blood relative to the electrolytes but not to the hemoglobin. The water diuresis would thus seem to fall under the general law that an increase in the concentration of any constituent of the plasma causes an increase in its rate of elimination by the kidney.

Certain difficulties, however, arise with this interpretation of water diuresis. Ginsberg (1912) compared the amounts of urine secreted after water and saline by mouth and after intravenous injection of the same solutions, and found that the diuresis produced by fluids intravenously was less marked than that produced by giving smaller quantities by mouth. Cow (1912, 1914) confirmed these findings and believed he had obtained evidence that the water diuresis was due to the extraction of a diuretic hormone from the intestine, but Hashimoto (1914) states that the results can be explained entirely on the salt content of the intestinal extracts. Priestley (1915–16) ingested Locke's solution in large amounts and obtained much less diuresis than with water. Adolph (1921), however, found this Locke's solution to be distinctly hypotonic to human blood, and upon ingesting large amounts of 1 per cent sodium chloride solution, he found practically no diuresis. This has since been confirmed by Baird and Haldane (1922), and also in this laboratory. Now, it is difficult to see why a dilute salt solution should not extract the hypothetical intestinal hormone just as well as distilled water. Moreover it should not change the osmotic pressure of the plasma as the ingestion of water has been found to do. It appears, therefore, that the stimulus to the water diuresis is the slight lowering of the osmotic pressure of the plasma. However, Adolph has found that if isotonic potassium chloride solution is ingested instead of sodium chloride, a good diuresis results, probably due to the fact that the large excess of potassium in the blood is excreted and in its excretion causes a diuresis.

Rioch, in some work on several men and dogs (as yet unpublished) confirmed the change in conductivity of the serum produced by the ingestion of water, but noticed that the maximum drop in conductivity did not parallel the maximum diuresis, there being a lag of about one-half hour in the diuresis curve. This is similar to the lag in diuresis noticed by Davis (1917) on injecting hypotonic salt solutions into unanesthetized dogs.

Very little increase in the solid constituents of the urine relative to the increase in water elimination results from the ingestion of water. Creatinine is unchanged in absolute quantity; urea and sulphates are increased but never more than doubled; ammonia and phosphates are generally slightly increased, but may be unchanged in amount (Shaffer, 1908; Marshall, 1920; Carr, 1921). The percentages of all substances in the dilute urine after water drinking are decreased. It has been found that chloride, bicarbonate and phosphate may be less concentrated in the urine than in the plasma, while urea and sulphate always occur in the urine in a greater concentration than in the plasma.

In terms of the theory which we have tentatively adopted, the water diuresis must be brought about mainly by an inhibition of the reabsorption of water by the tubule cells, since urea and sulphate are not increased in absolute amount to any great extent. At the same time one must assume that all of the glucose and a large part of the chloride are reabsorbed from the glomerular filtrate.

Another phase of the chemical regulation of urine secretion is the effect of a change in the composition of the blood plasma on other substances in the urine than those which have varied in the blood. In regard to water excretion, it is probably correct to state that the presence in excessive amounts of almost any diffusible body in the blood produces marked changes in the rate of elimination of urine. Just as in water diuresis we have assumed that the lowering of the osmotic pressure of the plasma is the stimulus to the kidney, so in salt diuresis, we may assume that an increased osmotic pressure acts as a stimulus. It appears probable that the diuresis depends mainly upon the number of dissolved particles introduced into the blood (Adolph, 1923). Acids and alkalis, when present in the blood in excess, as well as salts and diffusible non-electrolytes, cause a marked diuresis. Since there is a maximum concentration that can be reached by any substance in the urine (Ambard, 1920) and probably also a limit to the total molecular concentration of the urine (Davies, Haldane and Peskett, 1922; Adolph, 1923), this type of diuresis may be caused by the excretion of the neces-

sary water to eliminate the substance present in the body in excess. At any rate, it would seem to be mainly due to an osmotic hindrance to reabsorption of water in the tubules, but may also involve a somewhat increased filtration.

Another change in the composition of the blood which should cause a definite increase in the rate of water elimination is a dilution of the colloids of the plasma. This should directly increase the effective filtration pressure at the glomerular capsule. Very few facts can be found, however, to aid one in deciding what rôle this factor plays under normal conditions. It is, of course, certain that the injection of large amounts of fluid intravenously and the resulting hydremia must lower the protein content of the plasma, but the observations of Bakwin (1922) and Schultz (1923) do not lend support to any great influence, normally, of changes in colloid concentration.

Any of the changes in the chemical composition of the plasma which give rise to a diuresis will undoubtedly affect to some extent the rate of excretion of other substances than water. Changes in the acid-base equilibrium of the blood bring about changes in the rate of elimination of urinary constituents apart from changes in the rate of water output. Thus, an increase in hydrogen ion concentration of the blood causes an increased elimination of phosphates, non-volatile acids and ammonia (Wilson, 1923). A decrease in the hydrogen ion concentration may cause a marked increase in the rate of elimination of bicarbonate, even when the bicarbonate concentration in the plasma has been decreased (Davies, Haldane and Kennaway, 1920).

It remains to consider under chemical changes in the blood, the effect that the presence of hormones and foreign substances, such as drugs, may exert upon the activity of the kidneys. The effect of the latter can not be discussed within the limits assigned to this review, but the former must be given some consideration, although but little is known at present of their rôle in urinary secretion.

Some evidence has accumulated that the internal secretion of the pituitary body plays a rôle in regulating renal function, and definite effects on the urine are produced by the injection of pituitary extracts. The well known diuretic effect of these extracts on anesthetized animals is not believed to be their normal action, but is in all likelihood a conditioned response brought about by anesthetics or other factors (Abel, Rouiller and Geiling, 1923; Geiling, 1926). The antidiuretic effect of pituitary extracts, first observed and utilized in the treatment of dia-

betes insipidus by Von den Velden (1913) and confirmed since by many workers, is undoubtedly the normal effect in man and probably in normal unanesthetized laboratory animals. This antidiuretic action of extracts of the posterior lobe of the pituitary body can be best demonstrated in man or normal unanesthetized animals by an injection of the extract after a diuresis has been produced by ingestion of water (Motzfeldt, 1917; Houssay, Galan and Hegrete, 1920; Weir, Larson and Rowntree, 1922; Underhill and Pack, 1923; Fromherz, 1923; Molitor and Pick, 1924; Smith and McClosky, 1924). The injection causes a distinct decrease in the rate of urine elimination, but the diuresis occurs in a normal manner some hours later. Most observers believe that the antidiuretic action is brought about by a direct effect on the kidney (Oehme, 1921; Priestley, 1921; Craig, 1925; Miura, 1925).

That the internal secretion of the pituitary may exert an influence on the elimination of other substances by the kidney independent of its effect on water excretion is indicated by the investigations of Addis, Starling, and their collaborators. Addis and his co-workers (Addis, Barnett and Shevky, 1918) demonstrated a decrease in the rate of urea excretion from subcutaneous injections of pituitary extract. This they state to be independent of any influence on the blood urea or rate of water elimination. Starling and Verney (1925) find in the heart-lung-kidney preparation that the urine is always hypotonic compared to the blood plasma, this being due to an inability of this preparation to concentrate chloride or even, in fact, to raise the chloride concentration of the urine to more than a fraction of that occurring in the plasma. They believe this to be due to the absence of the internal secretion of the pituitary, since the addition of pituitary liquid to the blood increases the concentration of chloride in the urine. In a later paper, Brull and Eichholtz (1925) report that the removal of the pituitary gland or injury to the tuber cinereum, in anesthetized intact dogs, markedly diminishes or abolishes the elimination of phosphate as well as chloride in the urine.

The only other endocrine glands for which any evidence of a connection with renal activity exists are the adrenal bodies. Marshall and Davis (1916) found a definite lowering of the excretory efficiency of the kidney in adrenalectomized cats, and, moreover, state that this may occur with a normal blood pressure, and when the animals are in excellent physical condition. Addis and his collaborators (Addis, Barnett and Shevky, 1918; Bevier and Shevky, 1919–20) also report that removal of the adrenals in rabbits decreases the efficiency of the kidney in elimi-

nating urea, and, in addition, that the injection of small doses of adrenalin into normal animals increases the urea excreting capacity of the kidney.

The voluminous literature on the action of the renal nerves may be summed up in the statement that, although many investigators (Jungmann and Meyer, 1913; Rohde and Ellinger, 1913; Asher and Pearce, 1914; Jost, 1914; Asher, 1915) believed that they had demonstrated secretory fibers to the renal cells, a careful survey of the evidence leads to the conclusion that, up to the present, the only definite influence of the renal nerves, which has been proven, is due to changes in the circulation of the organ brought about by vasomotor fibers (Marshall and Kolls, 1919; Pearce and Carter, 1915; Yoshimura, 1920). One kidney has been transplanted to other locations in the organism or reimplanted in its normal site, and appears to function normally without any nerve fibers when the other organ is removed (Löbenhoffer, 1913; Quinby, 1916, 1917; Dederer, 1918). These experiments certainly prove that the renal nerves are not essential for the maintenance of life, but do not rule out some specific function for the nerves on account of the difficulty in being sure that the function of the organ is entirely normal. This would demand much more careful studies of all the activities of the kidney than have been conducted.

Among the more recent investigators on the function of the renal nerves may be mentioned Mauerhofer (1917), Bellido (1917), Carnot, Rathery and Gerard (1921), Ellinger (1921), Hara (1922), Pico (1923), Kichikawa (1925) and Ellinger and Hirt (1925). The experiments of Ellinger and Hirt appear to present more satisfactory evidence of some direct influence of the renal nerves than has hitherto been obtained. Their study is based upon the anatomical researches of Hirt (1924) which have confirmed Jost's (1914) contention that fibers from the abdominal sympathetic run to the kidney. The consistent increase in the elimination of ammonia which they obtain on section of these abdominal sympathetic fibers with no change in the urine flow, if confirmed, would certainly seem to indicate that ammonia excretion is under nervous control. Their other conclusions, in regard to secretory nerves, are by no means as well supported by their evidence, but may be briefly mentioned. The minor splanchnics influence water and electrolyte elimination through their vasomotor action; the abdominal sympathetics have no influence on urinary volume, but inhibit ammonia formation, total acid and phosphate excretion, and slightly accelerate the total nitrogen elimination; the major splanchnic acts as an antagonist to the

abdominal fibers; and, finally, the vagus influences water excretion and inhibits the elimination of total nitrogen, but the mechanism of this is not clear. The histological investigations of Berkley (1893) and Smirnow (1901) demonstrating nerve fibers ending on and between the convoluted tubule cells must certainly be taken into consideration. To the reviewer it appears that a reëxamination of the question of secretory nerves would appear advisable, provided that attention is focused more upon the possibility of nervous impulses influencing the elimination of some of the solid constituents of the urine, either by changing reabsorption or secretion in the tubule, and less upon the elimination of water as has previously been done. In view of the apparent differences between the kidney of the lower vertebrates and that of mammals, it would also seem to be profitable to investigate the action of the renal nerves in some of the lower vertebrates.

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DIET AND STERILITY

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The problem of the causes of sterility and their remedy has always commanded a considerable amount of attention from the biologist, not only because of its intrinsic interest, but because of the strong influence it may exert in human affairs, and in the stockyard. There is another consideration which may seem of small moment compared with the others, but which has a great practical bearing for the scientific investigator, and that is the fertility of laboratory stock. The ability to reproduce and rear healthy young constitutes perhaps the best and most delicate test of an animal's physical fitness and constitutional vigor, and in animal experimentation it is of paramount importance that the stock should be a healthy one, so as to reduce the risk of waste and perhaps failure, and also to reduce the number of variable factors by assuring that the animals conform as nearly as possible to a mode of normality.

The vast amount of nutritional research done since 1915 has disclosed, amongst a mass of other invaluable data, facts with regard to reproduction which have revolutionized our ideas about it. The discovery of vitamins and the fact that deficiency diseases are the result of their absence, naturally stimulated curiosity with regard to their possible effects on fertility, and a close connection has been established, and indeed a new vitamin (E) has been separated out from the others as being specially associated with the generative function.

It has long been recognized that nutrition has a great effect on reproduction, both in the human and in the lower animals, as is borne out by the general practice of stock breeders of giving breeding animals extra rations. It is usual to pay more attention to the male in this way, however, which certainly is attended by a measure of success, but is not the most efficient scheme, although it may be indicated by considerations of expense. Moreover it occasionally defeats its own ends in the hands of less skilful husbandmen, for overfeeding (frequently combined with lack of exercise) has indubitally a detrimental effect on fertility. This will be discussed in more detail later. The females customarily do

receive special attention in one class of stock, namely, the hill sheep of Scotland, where "flushing" is resorted to. This consists of supplementing the normal diet of hill pasture with concentrated foods, oats, turnips, cakes and the like, for some three weeks before the annual oestrus or "tupping" season. Marshall (56) and Nichols (66) have both investigated this practice, and agree that there is a definite increase in fertility as compared with the untreated flocks.

A general proposition might be made to the effect that the capacity for reproduction is a function of the well-being of the animal, and dietaries which affect this general well-being either adversely or favorably, affect the function of reproduction in like manner. Indeed it is frequently the case that the first clinical symptom of impaired health or debility is the comparative or complete inability to reproduce. Reynolds and Macomber's valuable contribution to the physiology of reproduction (74), (75), (76) shows this very clearly. They found that moderate deficiencies of various sorts in otherwise excellent diets, e.g., decrease in fat-soluble vitamin, calcium, protein, iron or iodine, resulted in a decrease in fertility, and that fertility was not merely a function of the generative organs, but of the whole organism. From an examination of their breeding records, they discovered that the fertility of an individual could be evaluated, and an index assigned, and that the fertility of the mating was the product of the indices of the parents, so that having established the threshold for the capacity to reproduce, when the indices of two animals were known, it was possible to predict with certitude whether the mating would be fertile or not. In the particular stock the threshold was found to be 0.45, and when an individual of, say, 0.8 fertility index was mated to one of say, 0.7, then the product was 0.56, and the mating fertile, while if the second had an index of only 0.4, then the product was 0.32, and the mating sterile. This individual variation in reproductive potentiality is often obvious in domestic animals, and careful analyses of breeding records would probably show it even more markedly. There is some evidence that such variation may be genetic in origin, but it seems certain that the value assignable to it under normal environmental conditions may be elevated or depressed by suitable alterations of the surroundings. Darwin recognised this, and in his *Variation of Plants and Animals under Domestication* gives a great number of facts which substantiate the conception. The principal variations in the environment are food, exercise and temperature. With regard to exercise it will suffice to say that Reynolds and Macomber (75) record that lack of exercise resulted in a diminution

of fertility in rats; Hartman (30) found that exercise lessened the atresia folliculi which occurred in his opossums, although their diet was an adequate one, and that Stieve (86), working with hens, induced the degeneration of ripe oöcytes by removing the birds from large runs to small pens.

Further, Reynolds and Macomber found that by their various deficiency diets it was possible to lower the fertility index, so that the matings could be made sterile without interfering with the general health and appearance of the animals. This has a very obvious bearing on such experiments as the testing of the fertility of females under a special regime with normal males. They also report cases of depressed relative fertility in human marriages which responded favorably to a change of diet (76).

The possible ways in which reproduction in the female can be affected by diet are: by a specific action on the ovary, *a*, by inhibiting the production of healthy ova; *b*, by causing atresia; *c*, by interfering with its endocrine functions; or *d*, by causing an atonic condition of the uterus; *e*, by affecting implantation, or *f*, the placenta or fetus.

Leo Loeb (45) found that if guinea pigs were fed with a small quantity of oats and grass, which is a fairly adequate qualitative ration for this animal, the resulting inanition caused a delay or absence of ovulation depending on the period of exposure to the unfavorable conditions. None of his animals became pregnant, and one, already pregnant at the start of the experiment, aborted. Histological examination showed that the cells of the membrana granulosa degenerated, followed by atresia folliculi, and in the most advanced case (43) the connective tissue was affected, having become shrunk and inactive, with a resulting concrescence of the follicles. This hypo-typical condition is marked by the occurrence of small to medium sized follicules, when large ones should be present, and it is a reasonable presumption that the ova themselves are affected, for Walsh (94) has shown that the ova exert a stimulus on the granulosa cells, and when pathological conditions lessen the growth energy of the follicle, the granulosa degenerates. Inanition of from six to thirty-three days brought about these changes, but in a degree which varied unversely with the weight of the animals, for as they approached full size more nearly, so they were more resistant to the ill effects. The mitochondria of the follicular cells and of the ovum are much diminished and deformed, subsequent to long fasting, according to Russo (79) who investigated the question in rabbits, and eventually the ova and follicles become shrunken and atrophied. This obser-

vation is important, because the condition of the mitochondria may be regarded as a useful measure of the integrity of cellular function.

These results are not unexpected, but they have a bearing on other considerations. The grounds for accepting Loeb's theories (44) of the sensitizing effect of the corpus luteum on the uterus, and the control of the mammary gland by the ovary, are strong, and hence it is obvious that malnutrition could affect these organs indirectly, even if it did not do so directly. Papanicolaou and Stockard confirmed this work on the guinea pig, using carrots as the sole diet, and observed underfeeding late in dioestrum was more effective than when early, and brought about a prolongation of this state, circulatory congestion, wholesale degeneration of follicles, and suppression of oestrus; while Evans and Bishop (18) had the same results with rats.

Oestrus appears to be particularly sensitive to vitamin A deficiency; indeed, Evans and Bishop (18) consider it to be the most delicate test for the vitamin, its deficiency causing a prolongation of the desquamative oestral changes, while a deficiency of B obliterates oestrus. Both these results were confirmed by Hoffman (32). The first two workers also found the oestrous cycle to be disturbed by an absence of fat from the diet, insufficient or poor quality protein, or small salt content (18). In many metabolic experiments the sensitivity of oestrus to nutritional disturbances appears very clearly, showing delay, irregularity or disappearance with even slight variations. Hart and Steenbock (24) found this with swine on diets of grain only fortified with common salt, and the calcium oxide of the naturally hard water of the district. In this case the low calcium intake appears to have been the main fault. This was also shown to occur in cattle on a ration restricted to the wheat plant, by Hart and his co-workers (22). Here the low salt intake was complicated by a toxic factor inherent in the wheat, which is also described by Hoffman (33). The oat plant, too, was found to be an inadequate source of nutriment for cattle (25).

Since reproduction is one of the first physiological functions to be influenced by inanition, it might be expected that the ovaries are among the first organs to suffer macroscopic change, and this was found to be the case in the rat by Jackson and Stewart (34), but the testes are apparently not so sensitive. Mattill and Conklin (60) also noticed this with their feeding of rats with fresh milk, and with 55 per cent dry whole milk—40 per cent starch—5 per cent butter fat.

The restriction of diet may be quantitative or qualitative, as shown by Slonaker and Card (85) who found that a diet similar to the vege-

tarian régime adopted occasionally by man, caused a delay of pubescence in the rat and increased the incidence of infertility four times, and still further in the second and third generations, when the greater number were sterile. Fertility, however, was restored by a resumption of the omnivorous diet of a type approximating that of man.

It has been said that pathological conditions may give rise to malnutrition, and subsequent derangement of the reproductive function, and such states are legion. As a type alcoholism may be taken. It might be argued that alcohol is a poison, but since it is regarded as a natural beverage, even indeed a food, by a large section of mankind, it is relevant to consider it here. Stockard and Papanicolaou (87) give an account of an extended series of experiments, which they conducted with guinea pigs, and they state that a profound influence is exercised on the germ cells by continual administration of alcohol. The weakest are destroyed, and those that survive form zygotes which may be seriously injured, and die, so that there is an increased prenatal mortality, and a consequent decrease in litter size. The effect is cumulative, influencing the descendants, as was also the finding of MacDowell (54) who showed that there were two influences at work, an inherited modification which reduces the size of the litters, and a selective elimination of germ plasm factors detrimental to litter production, that is, of zygotes homozygous for sublethal allelomorphs. Thus there is a braking tendency on the rate of reduction. The same worker, with Lord (55), states that the reduction of litter size in heavily alcoholized mice is not due to a lessening in the number of ova, but to an increased prenatal mortality. In the case of man, ovulation is probably interfered with, as Gibbons (20a) points out that amenorrhea is frequently associated with alcoholism, and there appears little doubt that amenorrhea is intimately connected with the absence of ovulation.

An interesting possibility suggests itself, namely, the relationship of diet to "war sterility." Several Continental workers have observed the special occurrence of amenorrhea and sterility during and after the World War, and also an increased incidence of exophthalmic goiter, with accompanying diminution in the size of the uterus (Blondel, 5; Ebeler, 13; Vaerting, 93). It is probably true that emotional factors played some part in the etiology of the goitre, but there is a strong likelihood that much of the war amenorrhea was due to the insufficient and badly balanced diets of the suffering countries. In any case the condition was worst in Germany and Austria, where the food supplies were also the most unfavorable. Zweifel (100) directly attributes the

cessation of menses to the low diet, and notes further that the average duration of pregnancy was increased by three or four days. Evidence regarding the effect of inanition on thyroid function is scarce, but there is a great deal regarding the interrelation of thyroid and ovary, e.g., Goodall and Conn (21) and Brinizer (6): the former authors point out that thyroid function is in a measure under the guidance of ovarian activity. Thus a possible mechanism for the syndrome is suggested, namely, the interaction of the three factors, diet, ovary and thyroid. McCarrison was of the belief that deficiency of vitamin B accounted for the sterility, by causing ovarian atrophy, and quotes Vedder to the effect that amenorrhea is a symptom of women suffering from beri-beri. Scheffzek (80) is also of the opinion that war amenorrhea and sterility are due to vitamin deficiency and to disturbance of calcium and sodium metabolism. He found that there was an increase in dysmenorrhea, hyperemesis gravidarum, and primary uterine inertia ascribable to these deficiencies.

It is clear that such interference with the ovaries, as sketched above, will also result in a disturbance of its endocrine function, and hence on reproduction as influenced thereby. It must be admitted that though much regarding the incretory activity of the ovary is controversial, the condition of the uterus is dependent thereon, and also the fixation of the embryo in the early stages of nidation. While this is true, however, a direct influence may be exerted on the uterus by dietary deficiency. It is obvious that inanition, as it affects the weight and efficiency of all organs, will affect the uterus, but there is a strong possibility that the reaction is even more delicate. The demands made by a pregnant uterus are considerable, and if they are not satisfied it is reasonable to suppose that the uterus will fail to adapt itself to the conditions. For example, Campbell and Watson (7) and Watson (95) record that sterility is a common appearance in rats fed exclusively on meat, and that if such a diet is fed from the time of weaning, fibrosis and atrophy of the uterus follow. If, however, the commencement is postponed till about the age of ten to twelve weeks, this effect is not so marked. Atonicity of the uterus was a feature of cattle fed on poor roughage, with consequent pre-births, and failure to clean (Hart, Steenbock and Humphrey (25), (26)). In Kennedy's experiments on diets deficient in calcium (36) ovulation took place but the animals were sterile; and the uterus was pale, flaccid and microscopically in a state of inactivity. In no case was it possible to demonstrate signs of fetal resorption, although it may have occurred at a very early stage, or the ova might not have been

fertilizable. This deficiency of uterine tone due to calcium lack had been pointed out by Oliver (67) who stated that the uterus was very sensitive in this respect. Deficiency of potassium, magnesium or phosphorus might also cause diminished tone and resultant abortion, the mechanism being that the uterine wall does not oppose the irritable villi efficiently, and recedes from contact so that an imperfect coaption of the fetal and maternal tissues probably results. This divorce is apt to cause rupture of the rapidly enlarging and thin-walled serotinal vessels, and hence abortion.

It is well recognized that prenatal death is so widespread an occurrence that Robinson (78) has called it normal. It is probably due to a great variety of agencies, such as genetic lethal factors, toxic states and so on, but a certain amount at least may be assigned to dietary abnormalities. Hammond (28) says that the production of a large number of eggs per ovulation as occurs in the domestic rabbit compared with the wild variety, frequently outstrips the nutrition available for them, and leads to atrophy either as newly fertilized, or partly developed ova. Further (29) that fertilization during lactation results in all the fetuses of the batch being absorbed owing to the lack of uterine nutrition. Parkes corroborated this with mice (71), finding that the proportion of the fetuses which atrophy is increased by suckling. Kirkham (38) also working with mice found that the gestation period was prolonged if instituted during lactation, and if this is due to the diminution of available nutrition, it would bring it into line with Zweifel's findings on the prolongation of pregnancy in the human (100). It is recognized that the demands of the developing uterus and embryo take precedence over those of the maternal tissues, which in fact may themselves become depleted owing to the insistence of the fetus, as is shown by the incidence of dental caries and osteomalacia during pregnancy on a low calcium intake. The lactating mammary gland also appears to take precedence in its call on the supply of food, and it would even seem to come before the embryo. There is the possibility, however, that mammary secretion exerts a chalonic influence on the ovary, either by endocrine activity, as is maintained by some, or by nutritional selection which partially eliminates the supply of some essential substance or substances from the ovary. One aspect of this theory has been elaborated by Hammond, from Heape's conception of a "generative ferment," and it is extended to explain many other phenomena of reproduction in the female. It has a disadvantage in that it appears to multiply the postulates unnecessarily, and hitherto has not led to any proof as to the nature of the "ferment"

There appears to be a point at which further calls cannot be made on the maternal tissues, and the fetus has to bear the brunt of the deficiency. The absence of some special complexes affects the fetus even more quickly and seriously, such as those which cannot be elaborated by the mother, for example, the vitamins (84) or lysine (23). These deficiencies may result in death of the embryo and resorption, or absorption, or in natal or neonatal mortality. The last in a common phenomenon in inanition experiments, and has been reported as a sequela to many unbalanced diets. To mention only a few, Sherman and MacLeod (82) and Korenchevsky (40) found high or complete mortality of the few young born on a low vitamin A régime; Hart and his co-workers (22), (24), (25) with cattle and pigs had increased natal mortality from the restriction of the diets, one of the chief factors being the reduced salt content; Mattill and Stone (61) failed to rear young when the mother rats were fed various milk diets as the source of protein and vitamin; and Mattill and others (58) found that milk rations with a high fat content, fortified with 10 to 15 per cent wheat embryo, as a source of vitamin E, resulted in a high mortality of the young.

It will be realized from the above, how many are the vicissitudes to which the developing embryo may be exposed, and that while a certain amount is known, little is definite regarding the mechanism of the effects. The subject is an important one both for its scientific and practical interest, and it is one which will well repay further research.

Before passing to the discussion of specific food components, a word may be said on obesity. Stockmen are familiar with the fact that show condition is inimical to efficient breeding, and physicians are well aware that the incidence of sterility is higher among obese than among normal people. Why this should be is obscure, though it is certain that some cases are endocrine syndromes (myxedema, hypopituarism, etc.), and as such out of our province, but these are, in any case, relatively few. Exogenous obesity, in which there is an excess of intake over the output of an otherwise healthy individual, is commoner, and is not infrequently associated with impotence or oligospermia in the male, and with complete or relative amenorrhea or irregular oestrus in the female; in broader terms, with decreased or absent fertility. This condition may be cured by proper diet and exercise, as Kinsley (37) points out in regard to pigs.

Ovarian function seemed to be deranged by the conditions which cause obesity, although it has been maintained by some that the reverse occurs, but the evidence is not so good. Prostner (quoted by Dietrich,

12) never observed subsequent fattening in 101 ovariotomies on women, and Luthje concurred with this, and although Schickele found one-third of his cases put on weight, he ascribed this to other causes. In the human the conditions which give rise to exogenous obesity are not so active as they are in domestic animals of the show class, and only 2.52 per cent of women complaining of infertility show obesity as the sole cause. This figure is compiled from the statistics of several authors representing some 5,000 cases of sterility. In the case of domestic animals a confusing circumstance arises, as Williams (97) has noted, for while obesity causes sterility, the reverse may be true, for if a cow is sterile owing to say, a persistent corpus luteum, she is not doing any work, and becomes fat. Therefore it is important to preclude other causes before deciding that the true etiology of the infertility is obesity.

Marshall and Peel (57) and Gaff (quoted by Dietrich) believe that extreme fatness blocks the Fallopian tubes by mechanical pressure, or that fat infiltration of the ovary hinders ovulation. This is not accepted by Dietrich (12) and many others, and in the course of ovariotmy and autopsy of extremely fat rabbits the writer has not observed any condition in which pressure on the tubes appeared to be likely to effect sterility. It must be realized that while considerable fatness militates against fertility, it may not prove an absolute bar; Kritzler (42) indeed reports a cases of unusual fertility associated with great obesity. The relation of social status in man to fertility involves a great number of considerations, but there seems little doubt that dietary influences play at least some part in its determination; a fuller discussion of these questions will be found in the writings of Engelman (15), Heron (31) and Carr-Saunders (8).

The special components of the diet now call for consideration, and of these the protein element claims first place. It is an absolute necessity for growth and reproduction, since it is the only source of nitrogen for the body, and it requires to be fed at a certain level of intake, and to be "good" in quality, i.e., it must be able to supply the necessary eighteen or twenty amino acids. That the quality of the protein is very important has been shown by McCollum and his associates (50), (51) for example, oat protein being inferior to that of maize or wheat. Simmonds (84) also discusses the relative and supplementary values of protein in reproduction and lactation. The general belief that high protein content in a diet induces infertility is supported by the findings of Reader and Drummond (73), and Kennedy (36), and others including B. P. Watson (95) and D. Chalmers Watson (96), who used excessive

meat rations. It is possible, however, that these last were otherwise deficient, and the question is not yet settled for Simmonds (84) in a long and careful research, found her rats to be fertile on high protein levels (67 per cent), the source being meat protein. Probably there is a maximum limit above which sterility follows, for the writers above mentioned used higher levels of intake than Simmonds.

Fats are of less importance, and reproductive efficiency may be maintained on a quantity only great enough to supply the fat-soluble vitamins, if the ration is otherwise adequate. It is believed by Mattill (58) and Nelson (65) and their co-workers, however, that an excess of fat inhibits fertility. One may note in passing that Morant (64) has found lecithin to have a favorable influence on fertility, either pure or in vegetable form; he also states that the sex ratio is altered in the direction of a greater number of females, but one feels that more extended data are required on this point. Carbohydrates *per se* are not requisite for the exercise of the generative function, if the energy is supplied by protein and fat, but such a régime is too expensive for ordinary use, and prolonged feeding of protein in itself reduces fertility, and may affect the kidneys. Carbohydrate has an important function in supplying roughage, which mechanically aids digestion and tone of the intestinal muscles. Mitchell (63) found that the addition of agar-agar to purified diets removed intestinal statis, and permitted breeding in the rats, which had failed hitherto. Sure (91) and Kennedy (36) disagree with the necessity for this. The mineral content is of the greatest importance in reproduction as well as growth, and comparable to the protein component it requires to be adequate in variety as well as quantity, and must include Ca, K, Na, Mg, Fe, and P, S, Cl and I. Hart and his associates have done considerable work on the mineral deficiencies of foods ordinarily fed to cattle and pigs, and have found that both the wheat and oat plants had insufficient salt content, and that corn stover from moderately acid soils was lacking in lime. This last seems to be specially important for reproduction, as was also shown by the results of Kennedy (36) that a deficiency of calcium in a carefully balanced, and otherwise complete ration, caused sterility. This may be a specific effect on the ova or on the zygote. Korenchevsky and Carr (41) state that irrespective of the mother rats' diet, nearly the same amounts of calcium, phosphorus, nitrogen and water were found in the young; and it is perhaps a reasonable deduction that while the mother will supply these from her own tissues as far as possible, if she fails to supply enough, death and resorption of the embryo follows. Excess of salt

content prevents reproduction; the inhibitory level was found to be 5 per cent by Sure (89).

The dietary value of milk has been extensively investigated, and it is agreed that it does not support reproduction by itself. This is not due to lack of protein, or vitamins A, B or D (Sure, 89), or to iron or butter fat (Mattill and Conklin, 60), but to the low amount of inorganic constituents, according to Daniels and Hutton (10), who discovered that milk diet supplemented by the ash of lettuce, yeast or soy bean allowed of the production of young. An unexpected and inexplicable result of some of their experiments was that a milk ration fortified by the addition of some mineral substances found therein in small quantities, viz., manganese, iron, aluminium and sodium silicate admitted of the rearing of young for five generations, although they failed to improve the synthetic milk. This is a very interesting result, but its significance is obscure. Traces of iodine seem to be essential also, and in experiments with purified rations, traces of potassium iodide can conveniently be added to the drinking water. In large amount this element inhibits fertility (Adler, 1; Jastram, 35).

The relationship of vitamins to reproduction has attracted a large number of investigators, with valuable results, and it is now recognised that these are as important for the generative function as for growth; and indeed, in the case of rodents at least, a vitamin has been discovered which is essential for reproduction, and named variously X or E, but the latter name appears to be the best.

The mode of action of vitamins is unknown; it has been suggested that they are similar to enzymes, but at present this can only be regarded as a hypothesis designed to stimulate research, rather than to explain. Cramer (9) has made an important contribution by showing that they possess a positive stimulating, drug-like action, for if a diet which, though adequate, has a low vitamin content, is fortified by a rich supply of vitamins, an improvement follows which indicates a condition of vitamin underfeeding under the other régime. Sherman and Campbell (81) observed that additional milk in a ration already shown to be sufficient, allowed of better growth, increased fertility, and longer duration of the reproductive life. This may be due in part to the added protein, etc., but probably not less to the increased vitamin. The same worker with MacLeod (82) found that a deficiency of vitamin A, which did not interfere with growth, caused sterility. Korenchevsky's (40) and Eckstein's (14) work confirms this. Several suggestions have been made regarding the relationship of plant carotinoids to fat soluble vitamin, but

Palmer and Kennedy (68) have successfully proved that this idea is unfounded.

The investigations on water soluble B are less in amount, but the effect of deficiency in this in producing amenorrhea has been mentioned above; Hoffman (32) observed atrophy of the sexual organs consequent on a lack of this vitamin. This was also the finding of Parkes and Drummond (72) with male rats. There is no evidence available as to the effect of vitamin C, or anti-rachitic D (not the vitamin of Funk) on reproduction. The first has little or no action on the rat, which is the favorite animal for these experiments, but it is conceivable that it may be of importance in other forms, while the second has such a similar distribution, and physical properties to A, that so far no distinction has been drawn as to their effects in this respect.

Hogan and Harshaw (33a) found their diets supported reproduction but were deficient for lactation, which they point out is a consderably greater strain on the mother than gestation. The first rations contained 4 per cent dry yeast and 12.5 per cent butter fat, and few young survived more than a few days; this was altered to 6 per cent yeast, 10 per cent milk fat and 5 per cent cod liver oil, and the proportion of surviving young was increased; they were, however, subnormal during suckling. They regard the nature of the deficiency of their diets as still an open question, and though their diets were not very different from those of Evans and Bishop they do not consider that their findings necessarily support or contradict Evans and Bishop with regard to vitamin E, since their rations may not have been sufficiently purified to remove this vitamin, and their butter component may have been richer in it—which, indeed, is not unlikely since considerable quantitative variations occur in this product.

Vitamin E remains; as yet it has hardly emerged from controversy, but it is impossible to deny that the evidence in its favor is very strong. The existence of a vitamin necessary for reproduction was first described by Evans and Bishop (16) in 1922, when they found that rats fed on a purified diet were for the most part sterile in the first generation, and wholly so in the second, the failure to breed being due to a peculiar disease which affected the placenta and products of conception, resulting in their death and resorption. Ovulation and fertilization were not affected. By a process of elimination the onus of responsibility was removed from the known vitamins, and other components of the diet, but certain foodstuffs effected a cure. These were lettuce leaves, fresh or dry, egg yolk, meat (ox cheek and liver), wheat embryo, oats,

alfalfa and milk fat, and it was therefore assumed that these foods contained a vitamin complex which was a necessary factor for maintaining the integrity of placental structure. The content in milk fat was definite but low. This factor which was named X by the discoverers, but which is now termed E, is not stored in the body for long periods, is resistant to ordinary cooking temperatures, insoluble in water, but extractable with ethyl alcohol, ether, and with benzene ether and acetone (Sure, 91).

This effect on the reproduction of the female was confirmed by Mason (47a) incidentally to his investigation on the sequelae to vitamin E starvation on the male. His standard diet (casein 18 per cent, starch 54 per cent, lard 15 per cent, butter fat 9 per cent, salt mixture 4 per cent, with tablets of dry yeast, alfalfa clover or dandelion as a source of water-soluble vitamin) was deficient in E and while it permitted of excellent growth, reproduction was never secured. It may be pointed out that E may not have been entirely absent, as it is present in alfalfa and may perhaps be present in yeast, but the quantities fed are not specified and were probably small, and inadequate to supply the necessary protection. Histological preparations showed that various degrees of progressive testicular degeneration resulted from exposure to this diet for increasing periods and ranged from an aggregation and degeneration of mature sperm, while the remainder of the spermatogenic process was normal, to the typical cryptorchid appearance. These changes were prevented by the addition of 40 grams fresh lettuce per diem to the diet, and further partial prevention was obtained by feeding lettuce after an exhibition of the basic diet for some time. It appeared that those tubules which were as yet unaffected were maintained in a healthy condition, but those in which the degeneration had commenced progressed to the extreme stage.

Nelson with Heller and Fulmer (65) considered that the postulation of a new vitamin for reproduction was unnecessary, since three generations of rats were reared on synthetic diets containing only yeast and butter fat as sources of vitamins, but the mortality of the young was high. Later with Anderegg (3) the conclusion is arrived at that if there is such a vitamin, it is present in air-dried yeast in amounts comparable to wheat embryo. This, however, does not make allowance for the content fed in the butter fat. Anderegg (2) had already asserted that the assumption of E was unnecessary, but as Sure (91) points out, his diets all contained a high proportion of butter fat, which is one of the E-containing substances. It would appear from a preliminary note by

Mattill and Congdon (59) that yeast does contain something in the nature of vitamin E. In addition they state that factor E, fed in the form of lettuce, does not completely fortify a milk diet, for though reproduction is improved the mortality of the young is high. It might be suggested that the salt content is at fault, for a very large amount of lettuce would require to be fed to adjust this deficiency in a milk ration.

Further investigations have shown this vitamin to be present in yellow corn, hemp seed, cottonseed and olive oils, but not in cocoanut, linseed or sesame (Sure, 90) and alcoholic extract of wheat embryo and green kale (Miller and Yates, 62). Supplee and Dow (88) found that the reproductive potency of dry milk stored in air over long periods is lost, and that it is retained by storage in deoxygenated air, which suggests that E is destroyed by oxidation. Sherman and his associates (83) observed that on a diet in which white bread supplied four-fifths and milk one-fifth of the total calories, normal growth took place but not reproduction, but when the milk was increased to two-fifths, then reproduction was normal. The assumption is that this increase supplied enough of the necessary vitamin. It must be remembered, of course, that the butter fat percentage in milk varies within wide limits, and also that it is quite a possibility—although there is as yet no experimental proof—that the vitamin E content of the milk is altered by the nature of the cows' diet. Knudson and Ramdles (39) added cholesterol to an extracted cholesterol-free diet, which was also deficient in E, and which only supported very deficient reproduction, with the result that the fertility was much improved. This points to much interesting speculation, but more work upon it is still necessary.

Throughout this review the emphasis has been laid chiefly on the female, partly because more work has been done on this aspect than on the male, and partly because the generative processes in the female are more complicated and delicate, and therefore more liable to derangement. It will be realized that the field is a wide one, but one which conceals much of great profit, for not only is the intrinsic interest very considerable, but it bears on many wide biological problems—in embryology, animal ecology, evolution, and indeed, a hundred more.

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THE RETICULO-ENDOTHELIAL SYSTEM

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In recent years great interest has been aroused in the system of cells which Aschoff and Landau (1913) grouped together under the designation of "reticulo-endothelial metabolic apparatus." A voluminous literature has arisen and the number of publications continues to grow without abatement. The ramifications of these studies have extended into almost every field of medical inquiry. Unfortunately there is by no means unanimity of opinion concerning the independence, functional unity, interrelationships, morphological characteristics, derivation, and nomenclature of the members of this system. For reasons to be stated later, it appears justifiable for the present to retain the name "reticulo-endothelial system" which has become deeply rooted abroad and has gained considerable vogue in this country as well. In the following pages an attempt will be made to review in as unbiased a manner as possible the observations of early workers who laid the foundation for Aschoff's monumental work, to present the current views pertaining to the structural and functional relationships of the component cells of this group, and to indicate the loopholes in the present state of our knowledge.

HISTORICAL. Fifty years ago Ranvier called attention to the phagocytic properties of the free cells of the connective tissues, especially of the *taches laiteuses* of the omentum. These cells were described as large branching elements which not only have the power of engulfing foreign material but serve as carriers of nutritive material which they deposit in the tissues by breaking off bits of their protoplasm. Because of this alleged function (in amphibia), Ranvier termed them "clasmatocytes" (κλάσμα, piece broken off, and κύτος, cell).

The next great advance was made by Metschnikoff who differentiated between the circulating ameboid cells of the blood, i.e. the polymorphonuclear leucocytes or "microphages," and the relatively fixed ameboid cells of the tissues or "macrophages" and emphasized the wide distribution of the latter and their highly phagocytic properties. He included among the macrophages the large cells of the splenic pulp and lymph

nodes, certain endothelial cells, including the Kupffer stellate cells of the liver, the neuroglial and nerve cells, and some of the connective tissue cells. Of the circulating leucocytes only the large mononuclears were included, the small lymphocytes having been specifically excluded. It is evident that this able investigator recognized, if only vaguely, the existence of a system of such cells, widely distributed throughout the organism. He clearly appreciated their importance not only in the phagocytosis of foreign material but also in inflammatory and immunity processes, attributing to them the formation of macrocytases, hemolysins, agglutinins and coagulins.

Marchand's chief contribution was his demonstration that the clasmatocytes are derived from mesenchymal elements in the connective tissue, especially in the adventitial sheaths of the blood vessels. He showed that in the presence of inflammation these "adventitial cells" became transformed into the macrophages described by Metschnikoff. He further stated that these cells are capable of producing non-granular and granular leucocytes and accordingly termed them "leucocytoid cells."

Maximow (1902) in his celebrated studies upon inflammation showed that the mononuclear ameboid cells, which he called "polyblasts" because of their diversity of structure, were constantly present in inflammatory tissue. He derived them in part from lymphocytes which had emigrated from the circulating blood and in part from mobilized local "resting wandering cells."

In the meantime, Mallory's classical studies on typhoid fever (1898) had demonstrated that the large phagocytic cells of the inflamed Peyer's patches, the mesenteric lymph nodes, the spleen and the liver, took their origin from the lymphatic or vascular endothelium. He also found these cells, which he termed the "endothelial leucocytes," in the pulmonary capillaries, the thoracic duct and the general circulation.

The introduction of vital staining marked the next significant advance in the study of the identification and distribution of these cells. Ribbert (1904) showed that the endothelial and reticulum cells of the spleen, bone marrow and lymph nodes, the Kupffer cells of the liver, the reticulum cells of the thymus and certain connective tissue cells were specifically stained by lithium carmine. The general vascular endothelium either took the dye in a feeble manner or like the muscle fibers, ganglion and glial cells, rejected it. Of the glands of external secretion only the liver and kidney exhibited staining of the epithelial cells (morphological evidence of excretion of the dye). Ribbert succeeded in demonstrating

that the same cells which were vitally stained were able to absorb other substances injected into the circulation, e.g., iron, lipoids, etc. Bouffard (1906) was the first to show that these cells could be stained *intra vitam* with the benzidine dyes.

These studies were followed by the comprehensive investigations of Goldmann (1909) who demonstrated with the aid of Ehrlich's newly-discovered dyes that the vitally stained cells were identical with the clasmatocytes of Ranvier, the macrophages of Metschnikoff, the adventitial cells of Marchard, the polyblasts of Maximow and the *cellules rhagiocrines* of Renaut.

In 1913 Aschoff made the first report of the exhaustive investigations of his pupil Kiyono on vital staining in normal and pathological tissues, and in the following year these results were published by Kiyono in monographic form. The latter introduced the term "histiocyte" (ἱστίον, tissue, and κύτος, cell) to designate the mesenchymal, mononuclear phagocytes of the connective tissues, described in detail their reaction to vital staining by lithium carmine and their behavior in health and disease.

Further contributions to the study of these cells by means of *intra vitam* staining with the acid colloidal dyes and other substances were made by Evans and Schuleman, Downey, Tschaschin, F. A. Evans, pupils of Aschoff and Kiyono, McJunkin, Foot, Permar, Nagao, Nissen, and others.

Morphology. Aschoff gives the following table of the staining reactions of the mesenchymal elements (somewhat modified):

1. The *endothelial cells* of the blood and lymph vessels. They absorb the dye only after repeated injections and then only in the form of very fine granules.

2. The *fibrocytes* or fixed connective tissue cells. They store the dye in variable degree after prolonged staining and also in the form of rather fine granules, but more readily than the endothelial cells.

3. The *reticulum cells* of the splenic pulp, the cortical nodules and pulp cords of the lymph nodes and ultimately the remainder of the lymphatic apparatus. These cells readily take the vital dye, staining more intensely than the fibrocytes but their staining reaction is much less rapid and pronounced than the members of the groups which follow.

4. The *endothelial cells* of the sinuses of the lymph nodes, the blood sinuses of the spleen, the capillaries of the liver lobules (Kupffer stellate cells), bone marrow, adrenal cortex and hypophysis react to the vital dyes more rapidly and intensely than any other members of the series.

5. The *histiocytes* of the general connective tissues. These cells stain almost as readily as those of group 4, especially when in a state of augmented functional activity.

6. The *splenocytes* or pulp cells of the spleen and the vitally staining *monocytes* ("endothelial leucocytes" of Mallory," blood histiocytes" of Kiyono) which have their origin from the tissue histiocytes (group 5) and the reticulo-endothelial cells (groups 3 and 4).

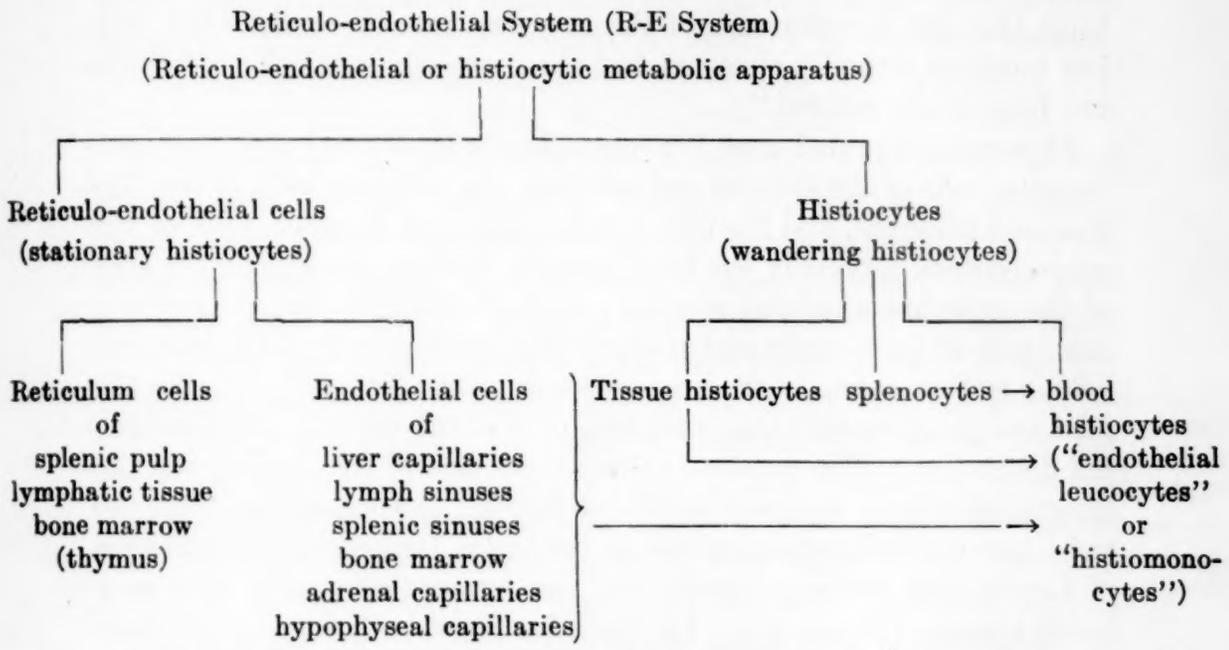
Reticulo-endothelial System (R-E System)
(Reticulo-endothelial or histiocytic metabolic apparatus)
Reticulo-endothelial cells
(stationary histiocytes)
Histiocytes
(wandering histiocytes)
Reticulum cells of splenic pulp lymphatic tissue bone marrow (thymus)
Endothelial cells of liver capillaries lymph sinuses splenic sinuses bone marrow adrenal capillaries hypophyseal capillaries
Tissue histiocytes
splenocytes
blood histiocytes ("endothelial leucocytes" or "histiomonocytes")

DIAGRAM FROM ASCHOFF (SLIGHTLY MODIFIED) (LECTURES IN PATHOLOGY, 1924, P. 16)

All but the common endothelial cells (group 1) and the fibrocytes (group 2) are included by Aschoff in the reticulo-endothelial system (see diagram). Mallory and his school as previously stated include all the endothelial cells but the justifiability of doing so is still disputed. There seems to be less reason for including the fibrocytes and for the present it is best to exclude them; yet it is best to keep an open mind concerning the potentialities of these cells. Recent investigations by v. Möllendorff indicate that the fibrocytes of the loose connective tissue of mice can be transformed into histiocytes. These studies if confirmed would indicate a closer relationship of the fibrocytes to the reticulo-endothelial system than has hitherto been assumed.

The term "reticulo-endothelial" was applied because of the supposed intimate relationship between the reticulum and endothelial cells of such organs as the liver and lymph nodes in which the endothelial cells

not only line the blood and lymph sinuses, respectively, but are believed also to manufacture the reticulum fibers (*Gitterfasern*). There is still much dispute concerning the relationship of the endothelial and reticular cells of the spleen (Weidenreich, Mollier, Neuberg, Cunningham, Krumbhaar), and lymph nodes (Downey, Heudorfer, Paschkis) and the exact status of the Kupffer cells of the liver (Schilling, Maximow, M. R. Lewis). Thus Downey and Aschoff[1] consider the reticular and endothelial cells of the lymph nodes to be identical, and Maximow maintains that the Kupffer cells of the liver are not true endothelial cells but modified reticular elements, but these are all questions which have not been finally settled.

Lubarsch suggested that the interstitial cells of the testis, the perivascular cells of the kidneys and adrenals, the reticular cells of the thymus and pancreas, and the iron-bearing glial and ganglion cells of the nerve centers, especially the basal ganglia, be included among the cells of the reticulo-endothelial system. Aschoff concedes that the perivascular cells of the kidneys and adrenals and reticular cells of the pancreas belong to this system in the broader sense of the term but excludes the glial and ganglion cells and reticulum cells of the thymus, all of epithelial derivation. The question of reticulum cells of the thymus and the interstitial cells of the testis is still *sub judice*. It appears that there are two kinds of vitally-staining cells in the testis, the true interstitial cells of Leydig and ordinary histiocytes, the former taking vital dyes in a feeble manner (Takamori). On the other hand both types participate in the storage of lipoids (Leupold). If included at all, the interstitial cells of the testis and the reticulum cells of the thymus would represent only an accessory branch of the reticulo-endothelial system. This is true also for the carmine-storing chromatophores of the skin the exact nature of which is still debated (Aschoff).

Aschoff disagrees with Mallory and his school (McJunkin, Foot, Permar) with respect to the development of free mononuclear phagocytes from ordinary capillary endothelium. According to Aschoff, only the specialized endothelium of the liver, spleen, bone marrow, lymph nodes, adrenal and hypophyseal capillaries belong to the reticulo-endothelial system, the vascular endothelium elsewhere being unable to produce mobile phagocytes. Lewis, in a study of the phagocytic cells

[1] In a more recent publication, Aschoff (Beihefte z. Med. Klin., 1926, XXII, 1) stated that the reticulum and endothelial cells of the lymph nodes are not quite identical, since they react differently toward particulate material, the former storing these substances more slowly and in less amount than the latter.

of the lungs in living frogs, was unable to detect any phagocytic activity on the part of the capillary endothelium, and Lang showed that the reaction of the common vascular endothelium to injected India ink is purely passive. Neither he nor Maximow was able to demonstrate any transformation of these cells into free phagocytes.

Maximow (1925) holds that the "polyblasts" arise in part from mobilized local histiocytes and in part from emigrated mononuclear leucocytes, both monocytes (of reticulo-endothelial derivation) and lymphocytes. This view is contradictory to Aschoff's in but one respect, namely the alleged transformation of lymphocytes into histiocytes, but in this respect Maximow differs from most other observers as well.

From a study of the mononuclear phagocytes (histiocytes) in the spleen, bone marrow, connective tissues, and peritoneal exudates, Sabin, Cunningham and their co-workers concluded that two types could be discriminated, monocytes and clasmatocytes. These cells not only react differently to supra-vital dyes (neutral red and janus green), but also appear to have a different embryological source, the monocytes being derived from primitive reticular cells and the clasmatocytes from endothelium. In supra-vital spreads with neutral red the monocyte exhibits a collection of fine bodies, which are probably vacuoles, arranged in the form of a rosette about a clear centrosphere and staining a fairly constant shade (salmon color). The clasmatocytes, on the other hand, also exhibit bodies which stain with neutral red, but these are vacuoles of digestion which vary conspicuously in size and color and are distributed without pattern in the cytoplasm. The contour of the nucleus is sharp and in vital spreads looks like the nucleus of fresh preparations after the addition of acetic acid (Sabin and Doan). The clasmatocytes correspond to the cells which exhibit the maximal reaction to the vital acid colloidal dyes, and are characterized by the large size of the foreign bodies which they engulf and by the lack of pattern in the cytoplasmic distribution of the engulfed material. The monocytes phagocytose less readily but are also capable of englobing both small and large structures, including blood-cells, but the presence of the rosette limits the position of the phagocytosed material to the periphery of the cell. Both monocytes and clasmatocytes possess motility.

Cunningham found in rabbits which had received intravenous injections of India ink that the endothelium of the venous sinuses of the spleen proliferated actively and produced large mononuclear phagocytic cells (clasmatocytes), which either remained attached, wandered out

into the pulp or were liberated into the circulation. Because of the sharp difference between the reaction of the endothelium of the splenic sinuses and that of the reticular cells of the pulp, Cunningham concluded that there is no such thing as "reticulo-endothelium," at least in the spleen. The evidence which he and his co-workers obtained was that the clasmatocytes are derived from the specific endothelium, but not reticulum, of the liver, spleen, bone marrow and lymph nodes in the adult, and from the general endothelium as well as the embryo. It is still an open question, according to Cunningham and Sabin, whether clasmatocytes may also be produced during irritations from the general vascular endothelium in post-natal life, as maintained by Foot, Permar, Herzog and others.

Tissue culture methods have furnished an additional means of studying the derivation of these cells. Lewis and Lewis showed that the large mononuclear cells in incubated blood become transformed into macrophages, clasmatocytes, epithelioid cells and giant cells. What then is the origin of the large mononuclear cells of the blood? On this question opinions are divided. According to Mallory and his followers, these cells are of endothelial origin, the common vascular endothelium as well as the specialized endothelium participating in their formation. According to Aschoff and Kiyono, there are three types of large mononuclear cells in the circulating blood: 1, the blood histiocytes, arising from the reticulo-endothelial cells of the liver, spleen, bone marrow and sometimes lymph nodes; 2, the transitional cells of Ehrlich, arising in the bone marrow and belonging to the myeloid series (monocytes of Naegeli); and 3, large mononuclear cells of lymphoid origin. McJunkin also recognized three types of large mononuclears: 1, the true monocytes, arising from the bone marrow and spleen; 2, the lymphendotheliocytes, arising from the lymphatic reticulo-endothelium; and 3, the hemendotheliocytes, arising from vascular endothelium.

Sabin, Doan and Cunningham (1924, 1925) in their studies on the monocyte, employed the supra-vital technique to differentiate the monocytes from other leucocytes. They believe that the monocytes arise from primitive reticular elements, which are found in abundance in the bone marrow and spleen and elsewhere. They present weighty evidence to show that the same reticular cell is the specific stem cell from which all the leucocytes arise, polymorphonuclear leucocytes, lymphocytes and monocytes. These studies simplify the classification and identification of the blood leucocytes. The differentiation between lymphocytes and monocytes can generally be made by means of the ordinary hemato-

logical stains, but may be accomplished with greater precision by means of the supra-vital technique. The large mononuclears and the transitional cells appear to represent different phases of the monocyte, the former being a younger stage of the latter (Sabin). The only blood cell which may cause confusion is the clasmatocyte which, according to Sabin and Doan (1926) is normally present in the circulation. The discrimination between monocytes and clasmatocytes can also be made by the supra-vital technique. In the light of these observations, there are four types of leucocytes in normal blood, polymorphonuclears, lymphocytes, monocytes and clasmatocytes. Sabin explains the apparent transformation of cultured monocytes into clasmatocytes (Lewis and Lewis) by the presence of clasmatocytes in the blood before it was incubated.

Mention must be made of Lewis' recent report (1926) which is revolutionary indeed. In a study of the macrophages (histiocytes) of the deep fascia of the thigh of rats in spreads supra-vitally stained with neutral red and janus green, he found them abundant in this location and, confirming his previous studies, was able to trace their development from the large mononuclears of the blood. He obtained no evidence of any transition from either connective tissue or endothelial cells in this locality. After inducing sterile inflammation by pinching the thigh, he noted a copious emigration of polymorphonuclear leucocytes, many of which appeared to change into monocytes and macrophages. In a further study, he confirmed these results in cultures of the buffy coat of the rat's blood. These observations will require convincing proof before they receive general acceptance.

The newer studies of Foot, Permar, Lewis and Lewis, and Sabin, Cunningham and their co-workers by no means invalidate the conception of the functional unity of the reticulo-endothelial system. All authors are agreed on the propriety of grouping together the endothelial cells of the venous sinuses of the spleen, the reticulo-endothelial cells of the bone marrow and lymph nodes and the Kupffer cells of the liver. Even though the exact relationship between the reticulum and endothelial cells of the spleen and some of the other organs is not settled, there can be no doubt that these cells exercise similar functions. With respect to the tissue histiocytes, all will welcome further information concerning their origin. Even the advocates of the dual origin (Sabin et al.) can not always differentiate between the two groups (monocytes and clasmatocytes) on the basis of function alone. The question of the participation of the common vascular endothelium in the production of free phagocytes

arises chiefly with respect to inflammatory reactions, and the final solution one way or the other will serve to terminate a controversy of long standing. Aschoff has clearly stated that there are many moot points with respect to the relationship between certain of the constituent cells of the reticulo-endothelial system, but what deserved special emphasis is similarity (not identity!) in the properties of these elements.

It is beyond the scope of this review to discuss in detail the large body of experimental and histological work which has been done to determine the origin of the alveolar phagocytes of the lung ("dust cells," *Herzfehlerzellen, cellules à graisse*). Despite the diligent efforts of numerous investigators to establish the source of these cells, the subject must still be considered *sub judice*. Theoretically these cells may arise from the alveolar epithelium, the pulmonary vascular endothelium, the histiocytes and the large mononuclear cells of the blood. The epithelial origin is supported by Aschoff and his pupils (*vide* Seemann, Ziegler's Beiträge, 1925, lxxiv, 345), the endothelial origin by Haythorn, Foot and Permar, and the histiocytic origin by Japanese investigators (*v.* Seemann), Lang and others. The literature has been amply reviewed by these authors. The cells in question are highly phagocytic, possess ameboid activity, and behave like histiocytes. Whether they should be included in the reticulo-endothelial system depends upon whether they are derived from reticular or endothelial cells or from alveolar epithelium. Studies with the supravital technique are now in progress (Gardner and Sacks).

A word about the Rouget cells to which Krogh and Vimtrop attribute the power of capillary contractility. Aschoff and Ohno take a contradictory view, asserting that these cells are related to adventitial cells (histiocytes). The studies of Clark and Clark on the capillaries of the tadpole's tail seem to settle this question in favor of Aschoff and Ohno's view, for they were able to demonstrate that endothelium is capable of contracting before the Rouget cells develop and that after they appear more contraction is initiated in areas in which no Rouget cells are present than in areas in which they are present. Thus it appears that the endothelial cell itself is the true contractile element.

Terminology. If we employ the term "reticulo-endothelial cells" to denote the reticular *and* endothelial cells without necessarily implying that these two types of cells are identical, there remains no valid reason why it should not be an acceptable designation for the collection of these cells in the spleen, liver, bone marrow and lymph nodes. The reticulo-endothelial system however also embraces the entire group of

tissue mononuclear phagocytes (histiocytes). If we accept the views of Cunningham and Sabin in separating the tissue histiocytes into two types, monocytes and clasmatocytes, and their derivation from reticular and endothelial cells respectively, it is clear that the term "reticulo-endothelial" as defined above is an eminently suitable name for these cells as well. The reticulo-endothelial system would thus consist of the reticulo-endothelial cells, i.e., reticular and endothelial cells, of the spleen, liver, bone marrow, lymph nodes, adrenal and hypophyseal capillaries and the histiocytes (clasmatocytes and monocytes) of the tissues and the circulating blood. Whereas the term "reticulo-endothelial cells" would serve as a collective name for these elements, the terms reticular cells, endothelial cells, histiocytes, monocytes and clasmatocytes could be used to designate the individual elements where such differentiation is possible. The term "clasmatocyte" is the least satisfactory of these names because of its etymology. It would complicate matters to attempt to introduce new terms, but greater clarity would have been gained if some such term as "reticulocyte" could be substituted for "monocyte" (indicating the reticular parentage of the latter) and "endotheliocyte" for "clasmatocyte" (indicating its endothelial derivation). The term "hemolyto-poietic," introduced by Krumbhaar, is a useful one to indicate the organs subserving hematopoietic and hemolytic functions, i.e. bone marrow, spleen, lymph nodes, and liver. These are the organs which are richest in sessile reticulo-endothelial cells.

HISTIOCYTOSIS; HISTIOCYTIC LEUKEMIA; SYSTEM DISEASES AND NEOPLASMS. Reference has already been made to the division of opinion concerning the types of monocytes in the blood and their derivation. A part of these cells at least appear to be reticulo-endothelial elements which have been left their position as sessile cells of the reticulo-endothelial system and entered the blood stream (Aschoff and Kiyono, Simpson). Normally very few of these cells are discovered in the blood (Aschoff), but in certain pathological conditions their size and number may be conspicuously increased and their cytoplasm laden with phagocyted material, e.g. erythrocytes, leucocytes, platelets, bacteria, pigment. Monocytosis or histiocytosis has been reported in typhoid fever (Eichhorst, Mallory, Holler), subacute bacterial endocarditis (Schilling, Bittorf, Hess, Seyderhelm, Libman and Rosenthal, Sampson, Kerr and Simpson, Joseph), paroxysmal hemoglobinuria (Ehrlich, Eason, Kämmerer and Meyer), malaria (Bushnell, Pappenheim, Schilling, Kartaschowa), typhus fever (Twerdynsky, Tuschinsky, Kartaschowa), relapsing fever

(Kartaschowa, Kartacheff), small pox (Schilling), cholera (Netoušek) and other conditions (Simpson). MacCallum (1903) observed such a large outpouring of the large mononuclear cells of typhoid fever from the thoracic duct into the circulation that a plugging of the smaller pulmonary arteries resulted. Van Nuys (1907) and Rowley (1908) each reported a case of fatal anemia with numerous large phagocytic cells in the circulation. The etiology was not discovered, but the history of these cases is strongly suggestive of subacute bacterial endocarditis.

Miller and Pepper noted intense phagocytosis of erythrocytes by "endothelial leucocytes" in the blood stream following the injection of typhoid bacilli into rabbits. Lipoid-containing histiocytes were found in the vessels issuing from the spleen and liver in rabbits with experimental hypercholesterolemia (Anitschkow), in the portal vein and certain arteries in Niemann's disease (Bloom), and in the peripheral blood in diabetes (Kawamura) and in rabbits following intravenous injections of aqueous suspensions of cholesterol oleate (Sacks).

Simpson produced circulating macrophages by chronic intravenous injections of colloidal dyes, India ink, and certain protein substances. She observed that the macrophages appeared in the blood in "showers," due to the sudden liberation of great numbers of these cells from the reticulo-endothelium of the liver and spleen especially. She further noted that there was a striking difference in the number of macrophages in the two ventricles, due apparently to the lodgment of these cells in the pulmonary capillaries. Schittenhelm and Erhardt later made somewhat similar observations. With respect to the relationship between the macrophages and the normal monocytes of the blood, Simpson concluded that the available evidence indicates a close biological relationship but not a proven identity. The experiments of Lewis and Lewis have definitely shown that macrophages can be formed in the blood itself from mononuclear cells, but according to the studies of Sabin and Doan clasmatocytes as well as monocytes are normally present in the circulating blood, and either of these cell types furnishes a possible source for the macrophages. The monocytoses should be restudied with Sabin's supravital technique.

In subacute bacterial endocarditis there is often a striking disparity between the number of macrophages in the blood of the ear and that of the finger. Hess concluded from histological examination of the lobe of the ear in patients who have succumbed to this disease that the phagocytes developed locally from the proliferating capillary endothelium.

On the other hand, there is evidence that the reticulo-endothelial cells of the hemolyto-poietic tissues are also in a state of proliferative activity (Schilling), so that it is possible that the circulating phagocytes of this disease have a dual origin.

It is not definitely known whether histiocytic leukemias exist. Monocytic leukemias have been described by Reschad and Schilling-Torgau, Rosenthal and Richter, but without supra-vital studies the diagnosis of the predominant cell remains uncertain. This statement is also true of Ewald's case. Clasmatocytes have been found in the blood in cases of myeloid and lymphatic leukemia (Doan and Sabin, pp. 823, 824), but the existence of true clasmatocytic (endotheliocytic) leukemia remains to be established. Cases of leukemic-like proliferations of the reticulo-endothelial cells of the hematopoietic organs but without leukemic blood have been reported by Goldschmidt and Isaac and Letterer. The cases of Borissowa and Pentmann also appear to fall in this group. A peculiar form of granulomatous proliferation of the reticulo-endothelial cells of the hematopoietic tissues was reported by Schultz, Wermbter and Puhl. Barth's case was one of myeloid leukemia in which the typical leukemic infiltrations of the hematopoietic organs were accompanied by extensive proliferations of the reticulo-endothelial cells, with the formation of atypical giant cells.

Neoplasms of various provinces of the reticulo-endothelial system, e.g. endothelioma of lymph nodes or spleen, have been described. Details can be found in Ewing's book. Lewis (M. R.) and Andervont in studies of Rous' chicken sarcoma and of Carrel's indol sarcoma by means of permanent sections, supravital spreads and tissue cultures showed that the malignant cells were hypertrophied mononuclear leucocytes which contained the infectious agent. Tumors could be produced in healthy chickens by inoculating them with the washed white blood-cells of tumor chickens. Lewis (W. H.) and Brüda cultured a tumor which developed spontaneously in a rat and noted that the only type of cell in the cultures was an epithelioid modification of the mononuclear blood cell. When cultures of these cells without the explant were implanted into another rat, a tumor similar to the original one developed. Since the latter had all the characteristics of a spindle cell sarcoma, these investigators consider it possible that some of the other tumors occurring both in mammals and in man may be white blood-cell tumors.

To what extent the reticulo-endothelial system is implicated in hematopoiesis in general is too extensive a subject to be considered briefly. Reference has already been made to the views of Cunningham,

Sabin and Doan whose studies indicate that a primitive reticular cell is the parent cell of the polymorphonuclear leucocytes, lymphocytes and monocytes. The same investigators (Doan, Cunningham and Sabin) have presented evidence to show that the red blood-cells are derived from the vascular endothelium of the bone marrow. The views of Kiyono and his collaborators and those of Maximow may be had at a glance by examining the charts in Aschoff's and Maximow's reviews, respectively.

RÔLE OF THE RETICULO-ENDOTHELIAL SYSTEM IN TUBERCULOSIS AND OTHER SPECIFIC INFECTIONS. From studies with vital dyes, Goldmann, Kiyono, Evans, Bowman and Winternitz and others concluded that the essential cell of the tubercle, the epithelioid cell, is derived from cells of the reticulo-endothelial system. Foot, investigating miliary tuberculosis and tuberculosis of the central nervous system, concluded that the tubercles in the lung, liver, spleen, kidneys, lymph nodes and omentum developed from proliferation of the local capillary endothelium, whereas in the nervous system the lesions were for the most part due to "endothelial leucocytes" which were carried to the affected areas by the blood stream. Foot employed India ink (Higgin's) to identify the capillary endothelium for which he asserts the ink is a specific stain. The evidence offered for the origin of epithelioid cells from ordinary capillary endothelium is not conclusive, and the endothelial derivation is specifically denied by Aschoff, Maximow, Lang, and Cunningham, Sabin and their co-workers. The latter group of investigators deny the origin of the epithelioid cells from the specific endothelium as well (*v. infra*).

Maximow, by making cultures of various tissues and of blood leucocytes inoculated with tubercle bacilli, showed that the epithelioid and giant cells have the same dual origin as the mononuclear exudate cells or "polyblasts" in common or purulent inflammation. He found that they arise in part from local fixed elements (histiocytes), and partly from emigrated or local, if available, mononuclear leucocytes, both monocytes and lymphocytes, the relative number of epithelioid cells of histiocytic or leucocytic origin depending upon the availability of these two types of cells. In Lang's experiments with lung cultures all the epithelioid cells arose from the tissue histiocytes, circulating leucocytes having been absent. On the other hand, the observations of Maximow on cultures of leucocytes inoculated with tubercle bacilli and those of Lewis and Lewis on incubated blood without bacterial inoculation conclusively demonstrate that the mononuclear leucocytes too can form epithelioid and giant cells. In this way are harmonized the older views of Baumgarten and Orth, on the one hand, who believed that the specific

cells of the tubercle developed exclusively from local fixed connective tissue elements, and those of the French school (Metschnikoff, Yersin, Borrell), on the other hand, who supported the leucocytic origin of these cells. Not all however are ready to accept the evidence which Maximow and Lang offer for the participation of the lymphocytes.

Cunningham, Sabin, Sugiyama and Kindwall in a careful study of the origin of the specific cells of the tubercle came to the conclusion that they are derived from the monocytes. To quote from these authors:

> We are now presenting evidence to show that the infection of tuberculosis causes an over-production of the monocyte, including all of its stages, namely, the reticular cell, the typical monocyte and its two derivatives, the epithelioid cell and the giant cell; that the tubercle bacillus so alters the cytoplasmic activity of the monocyte that the cell becomes a suitable medium in which the bacillus can live and multiply, or, in other words, that the organism of tuberculosis becomes a parasite within the cell; and that the marked overproduction of monocytes in the connective tissues is correlated, in the acute phase of the disease, with an increase in monocytes in the circulating blood.

According to these authors, the epithelioid cells arise either from the monocytes of the circulating blood, or from proliferation of local primitive reticular cells, which are found in abundance not only in the bone marrow and spleen, but also in the connective tissues of the lung and many other organs. The tissue monocytes are identical with the "histiocytes" from which Aschoff and Kiyono derive the epithelioid cells, but the latter investigators include under this designation both monocytes and clasmatocytes. Contrary to the opinion of a number of observers (Miller, Oppenheimer, Kiyono, Evans, Bowman and Winternitz, Foot), Sabin (1926) believes that endothelial cells and their derivatives, the clasmatocytes, do not form epithelioid cells. In the transformation of monocytes into epithelioid cells, in supravital studies, the typical rosette of the former is retained (in modified form) in the latter; on the other hand, there is no similar structure in the clasmatocytes from which the large rosette of the epithelioid cells may be derived. It is true that the Kupffer cells of the liver, which according to Sabin are true daughter endothelial cells, phagocytose tubercle bacilli and enlarge to form giant cells. But these giant cells, which may also be produced from the Kupffer cells by repeated injection of any particulate matter (Sabin and Doan), are different in the arrangement of their nuclei and in other respects from tubercular giant cells,[2] and there is no evidence that the Kupffer cells produce either Langhans giant cells or typical epithelioid cells (Sabin, 1926).

[2] In the Langhans giant cells, the nuclei are peripherally arranged because of the central position of the hypertrophied (monocytic) rosette (Sabin and Doan).

It has been demonstrated in fact that even in the liver the epithelioid cells of the tubercle are derived from reticular cells or monocytes (Sabin, Doan and Cunningham, 1925). Kageyama (Ziegler's Beiträge, 1925, lxxiv, 356) showed that the sinus endothelial cells of the lymph nodes were very active in phagocyting tubercle bacilli soon after they were injected into the peritoneal cavity, but that true epithelioid cells did not develop until a later period, when they were found to arise from the reticular cells. Dworski, Smith and Gardner, employing the supra-vital technique demonstrated that monocytes are the cells which are most active in the phagocytosis of tubercle bacilli in the peritoneal cavity of the guinea pig, the clasmatocytes playing a subordinate rôle. There can be no question that the endothelial cells (clasmatocytes) are implicated in the reaction of the organism to the tubercle bacillus, but the available evidence indicates that only reticular cells and their derivatives, the monocytes, produce true epithelioid cells and Langhans giant cells. The further development of the tubercle has been fully described by Gardner.

The rôle of the histiocytes ("endothelial leucocytes") in typhoid fever has been the subject of a careful study by Mallory and others (Gräff). The histiocyte is the essential cell of the lesions of sporotrichosis (D'Agata, Lawless), the lepra nodule (Chuma and Gujo, Herxheimer, Oliver), the specific lesions of Hodgkin's disease[3] (Aschoff) and of rheumatic fever (Aschoff, Sacks), rhinoscleroma (Mallory), and oriental boil (Oberling). Numerous histiocytes are found in the ulcerative lesions produced by the endameba histolytica (Foot) and in the lesions of malakoplakia of the urinary bladder (Landsteiner and Stoerk).

In malaria, the reticulo-endothelial cells of the liver and spleen are enlarged and contain phagocyted red and white blood corpuscles, pigment and parasites (Gaskell and Millar). Various yeasts and molds produce lesions in which histiocytes predominate. Proliferation of reticulo-endothelial cells is also found in kala azar, trypanosomiasis, typhus fever (Kuczynski), histoplasmosis (Darling; Watson and Riley) and Oroya fever (Noguchi). The ordinary capillary endothelium is observed to be in a state of proliferation in Rocky Mountain spotted fever (Wolbach, Todd and Palfrey), typhus fever, trench fever, measles (Mallory and Medlar) and heartwater (Cowdry), but whether these cells become mobilized as free phagocytes is still disputed.

[3] According to Cunningham, Sabin, Sugiyama and Kindwall (p. 274), the epithelioid cells of Hodgkin's disease stain with the supra-vital technique like those of tuberculosis.

RÔLE IN ORDINARY INFECTIONS. The reticulo-endothelial cells also participate actively in ordinary, i.e. non-specific infections. Their activity is manifest by the phagocytosis and disintegration of bacteria, their predominant rôle in immunity reactions (*v. infra*), their local proliferation and entrance into the blood stream (e.g. in subacute bacterial endocarditis), and their ingestion and destruction of red blood cells. The implication of these cells varies within wide limits; in certain instances it may be conspicuous and in others relatively slight. One of the earliest studies in this field is that of Wyssokowitsch (1886), who showed that bacteria injected intravenously were rapidly removed from the circulation by the fixed cells of the spleen and other organs. Observations upon the involvement of the reticulo-endothelial system in dysentery and other infectious diseases were made by Syssojew (1924), in chronic streptococcus infections by Siegmund, and in experimental staphylococcal and streptococcal infections of mice by Jacob. Domagk noted that the endothelial cells of the spleen, liver and lungs and the blood monocytes actively phagocyted and digested living and dead bacteria injected into mice. Amyloid was found at a very early period in the neighborhood of the phagocytic and proteolytic cells, especially in the liver and spleen.

THE RÔLE OF THE HISTIOCYTES IN LOCAL INFLAMMATION AND REPAIR. In local inflammatory processes and in repair the histiocytes act as scavengers and remove débris. In peritoneal and pleural inflammations, these cells form an important part of the exudate. On the other hand the mesothelial cells, which do not belong to the reticulo-endothelial system, play a subordinate rôle (Cunningham). The histiocytes, moreover, participate actively in the formation of granulation tissue. According to Maximow, the fibroblasts arise from the capillary endothelium and the polyblasts. Carrel and Ebeling and later Fisher were able to demonstrate the transformation of cultured monocytes into fibroblasts. This occurred when the cells were packed through mechanical factors preventing free migration (Carrel and Ebeling). In a recent communication, the latter reported that the inoculation of cultures of monocytes with filtered extracts of Rous' sarcoma frequently determined the appearance of fibroblasts.

RELATION TO BLOOD DESTRUCTION AND JAUNDICE. That the reticulo-endothelial system participates actively in the blood destruction is now well established. The simplest manner in which this occurs is in the phagocytosis of dying erythrocytes. According to Rous and Robertson, phagocytosis alone will not explain normal blood destruction,

at least in certain animals. These authors suggest that this process normally proceeds for the most part by fragmentation of the erythrocytes with subsequent removal of the hemoglobin-containing particles by the spleen and the bone marrow. Doan and Sabin confirmed these observations by showing that there is constant fragmentation of red blood-cells in the circulation of normal rabbits, the fragments being engulfed and destroyed by clasmatocytes (endothelial phagocytes) in the blood and tissues. In the increased hemolysis which accompanies certain infections, e.g., typhoid fever (Mallory, Gräff), Weil's disease (Lepehne), subacute bacterial endocarditis (Libman, Lubarsch), the spleen and other provinces of the reticulo-endothelial system are implicated to a variable degree. In a large number of hemolytic processes, e.g., infections, pernicious anemia (Eppinger), hunger and nutritional disorders (Okuneff, Lubarsch, Dubois), hemosiderin is found deposited in the reticulo-endothelial cells of the liver, spleen and other organs.

In normal mammals, the spleen appears to be the chief site of erythrocyte destruction. Mann, Sheard, Bollman and Baldes (1925) experimenting on normal animals showed by spectrophotometric methods that the blood issuing from the spleen and bone marrow contains more bilirubin (end product of hemoglobin disintegration) than the blood entering these structures. When the spleen is removed, the reticulo-endothelial cells of the rest of the hemolyto-poietic organs (liver, lymph nodes, bone marrow) rapidly compensate for the loss of this organ. Thus splenectomy in monkeys is followed by changes in the lymph nodes, bone marrow and Kupffer cells of the liver indicating that these structures take over the spleen's share of disposing of effete erythrocytes and their disintegration products (Krumbhaar and Musser). Similar compensatory changes following splenectomy which vary in different animals have been reported by a number of observers. Further details concerning the mechanism of erythrocyte destruction and the rôle of the spleen in this process are given in the excellent reviews of Rous and Krumbhaar.

In recent decades great strides have been made in the study of the pathogenesis of jaundice and the physiology of bile formation and the evidence is pointing more and more definitely to the reticulo-endothelial system as the seat of bile-pigment production. For many years it was widely taught as a result of Minkowski and Naunyn's experiments (1886) that the liver epithelial cells were responsible for bile pigment production. McNee (1913) who repeated their experiments showed that no marked jaundice results from intravascular hemolysis in hepatec-

tomized geese. From similar experiments, Minkowski and Naunyn had concluded that the liver and specifically its epithelial cells is essential for bile pigment production.[4] But the possible implication of Kupffer cells had to be considered as well as the epithelial cells. Inasmuch as in birds the bulk of the reticulo-endothelial cells is in the liver, it occurred to McNee that the absence of well-developed jaundice was due not to the elimination of the epithelial cells but of the Kupffer cells, the negligible amount of reticulo-endothelial tissue in the remaining organs being insufficient to compensate for their loss.[5]

During the same year, Whipple and Hooper working independently from McNee showed that ligation of all available vessels to the liver of dogs did not prevent the appearance of jaundice following the injection of hemoglobin. These experiments appeared to offer conclusive evidence of the extrahepatic formation of bilirubin, but Rich (1923) showed that the method of ligation adopted by these investigators did not absolutely exclude the liver from the circulation on account of anastomotic vessels in the diaphragm. The problem was attacked by Lepehne, Eppinger and others who studied the effect of "blocking" the reticulo-endothelial system. Lepehne found that it is possible to suppress almost completely the jaundice which ensues in birds after poisoning by arseniuretted hydrogen by saturating the reticulo-endothelial system with collargol injections. Rosenthal and Fisher and others were, however, unable to confirm this result. Later Elek apparently succeeded in confirming Lepehne's observations, but according to Rich his experiments are inconclusive. As Rich states, it is doubtful whether the function of the reticulo-endothelial cells can be entirely eliminated by the use of colloidal substances.

It has been known for a long time that hematoidin, which appears to be identical with bilirubin (Rich and Bumstead), is formed in blood extravasations (Virchow). The local histiocytes responsible for the phagocytosis of the red blood corpuscles also seem to be implicated in the final change from hemoglobin to bilirubin. Rich (1924) has shown that avian monocytes and tissues rich in histiocytes when cultivated *in vitro* are capable of converting hemoglobin into bile pigment.

The perfection of a method for complete extirpation of the liver in dogs by Mann, has made it possible to establish definitely that bile pigment can be formed extrahepatically (Mann, Bollman and Magath,

[4] This theory has recently been revived by Melchior, Rosenthal and Licht. For Aschoff's reply see Klin. Wochenschr., 1926, v, 482.

[5] The spleen is relatively very small in birds.

Makino, Rich). Bilirubin continues to be formed both spontaneously and after hemoglobin injection even if the method of operation does not involve a reduction of the blood supply of the liver prior to its complete extirpation (Rich; Mann, Sheard and Bollman). These findings establish the existence of a ready, extrahepatic mechanism for the manufacture of bile pigment, but they do not exclude the possibility that the liver normally produces the greater share of the bilirubin (though there is no evidence to this effect).

Jones and Jones ascribed the power of bilirubin formation to common endothelium. They induced intravascular hemolysis by immersing in ice water the arm of a patient suffering from paroxysmal hemoglobinuria. After a certain period bilirubin was discovered in blood drawn from this arm, which had been isolated from the general circulation by means of a tourniquet during the entire period of the experiment. Oppenheimer, however, repeated this experiment in dogs in all of its important details with negative results. The observations of McNee and Prusik also fail to support the theory of bile pigment formation by ordinary vascular endothelium. After perfusing a "heart-lung preparation" with laked blood or hemoglobin for six hours, no bilirubin was found in the perfusate.

The experiments thus far cited eliminate the liver epithelial cells and the common endothelium, and we may now state that there is no evidence that bilirubin can be formed in the blood stream, i.e., extracellularly, by the action of ferments (Rich and Bumstead). The negative experiments of Oppenheimer and McNee and Prusik also speak against the latter possibility. There is, however, much to show that bilirubin can be formed in organs containing an abundance of reticulo-endothelial tissue. Thus perfusion of the spleen with laked blood results in the appearance of bilirubin in the perfusate at the end of an hour (Ernst and Szappanyos). The formation of bile pigment by histiocytes in blood extravasations and in tissue cultures (Rich), and the "block" experiments of Lepehne also speak in favor of the reticulo-endothelial origin of bilirubin. Rich and Rienhoff have shown that in certain conditions the blood of the splenic vein contains more bile pigment than that of the splenic artery or other veins and Mann and his co-workers, employing the spectrophotometric method, have demonstrated that this occurs normally not only in the spleen but also in the bone marrow and the liver. On the other hand it is strange that Krumbhaar (1926) was unable to confirm this result for the spleen in a splenectomized case of hemolytic jaundice, but negative findings of course do not necessarily

nullify previous positive results. Discussing the conclusions of their investigations upon the site of bile pigment production, Mann and his co-workers (1925) state: "This is strong evidence that the theory that bilirubin is formed by the cells of the reticulo-endothelial system is correct, and that the cells of the same type in the liver can also form bilirubin." Further details can be found in their papers and in the excellent review of Rich.

LIPOID METABOLISM AND GAUCHER'S DISEASE. The hypercholesterolemia accompanying starvation leads to infiltration of the reticulo-endothelial cells of the spleen with cholesterol esters (Okuneff). Even normally, various lipoids may at times be stored in the spleen, especially in the reticulum cells. In diabetes, the Kupffer stellate cells of the liver are sometimes found to be infiltrated with doubly refractile lipoid and in a certain number of cases the reticulo-endothelial cells of the spleen become so hyperplastic and laden with lipoid material (cholesterol esters and other substances) that splenomegaly results (Schultze, Smith, Oppenheimer and Fishberg, Warren and Root). Lipemia has usually been present in the reported cases. Niemann was the first to report under the title *Ein unbekanntes Krankheitsbild* a nutritional disease of infants in which the reticulo-endothelial cells of the spleen and other organs were the seat of lipoid deposits not unlike those noted in diabetes. In the case reported by Bloom there was widespread lipoid infiltration of the reticular cells of the spleen, lymph nodes, and thymus, Kupffer cells of the liver, histiocytes in the connective tissue and in certain blood vessels, and large cells in the alveoli of the lungs. The changes noted in both diabetes and Niemann's disease are closely comparable to those seen in rabbits made hypercholesterolemic by the feeding of foods rich in cholesterol or cholesterol itself dissolved in oil. (Anitschkow, Chalatow, McMeans, Versé.)

Localized lipoid infiltrations of the histiocytes of the skin occur in the form of xanthoma or xanthelasma. They are generally associated with lipoidemia and are seen in liver disease, with or without jaundice, (Oberling, Schulte), diabetes mellitus (Major), pregnancy, certain forms of renal disease, and at times in cases in which the hypercholesterolemia is idiopathic (Arning and Lippman, Burns). The diabetic child reported by Oppenheimer and Fishberg had, in addition to xanthomata of the skin, lipoid infiltration in the spleen, liver and aorta.

Many authors have confused the general lipoid infiltrations of the reticulo-endothelial system with Gaucher's disease. In both, the reticulo-endothelial cells of the liver, spleen, bone marrow and certain

lymph nodes may be involved. But as Mandlebaum and Downey have shown, the Gaucher cells do not contain any of the fats or lipoids which are demonstrable by the usual microchemical methods. The careful histological studies of these authors led them to the conclusion that the Gaucher cells in the spleen are derived from the reticulum cells, but that participation of the sinus endothelium can not be absolutely excluded. Pick found a pronounced difference in the iron content of the endothelium of the venous sinuses and the Gaucher cells of the spleen, the former being heavily laden with iron in granular form but otherwise unchanged and the latter exhibiting slight diffuse infiltration or none at all. This finding and the discovery of arterioles in the midst of certain of the nests of Gaucher cells led him to the conclusion that only reticulum and pulp cells were the source of these cells in the spleen. In the liver, the Gaucher cells arise in the histiocytes of Glisson's capsule and in the adventitial and periadventitial connective tissue cells of the central veins of the lobules (Pick), whereas in the bone marrow and lymph nodes they arise from the reticular cells (Mandlebaum and Downey, Pick). The most promising chemical studies thus far are those of Epstein and Lieb who agree with Mandlebaum and Downey that cholesterol and its esters, neutral fat, fatty acids and lecithin can not be demonstrated in the Gaucher cells. Their investigations led them to the conclusion that the chief constituent of these cells is a complex substance of the nature of a cerebroside (sphingo-galactoside) and that phosphatids are also present. Thus it appears that Gaucher's disease is a system disease of the reticulo-endothelial apparatus associated with some disturbance of the lipoid metabolism, but of a different kind from that which leads to the deposition of cholesterol or fat in the reticulo-endothelial cells.

Relation to antibody formation. A great deal has been written to show the implication of the reticulo-endothelial system in antibody formation. In view of the widespread distribution of its constituent cells, their highly phagocytic properties and resistance, it is not strange that attention should have been directed to these elements in the search for the site of antibody production in both general and local immunity processes. Before the functional unity of this system was recognized, attempts were made to show that antibodies were formed in certain organs, e.g., the spleen, rather than in specific cells. Such experiments are more intelligible in the light of our present knowledge. Thus the effects of extirpation of the spleen appear to be the result of elimination of its reticulo-endothelial cells, and the variable effect on antibody for-

mation seems to be related to the variable degree of vicarious and compensatory activity in the rest of the reticulo-endothelial system. No attempt will be made to review the older literature; it is summarized in the papers to which reference will be made.

Cary studied the fate of foreign erythrocytes introduced into the circulation of rabbits and showed that the antigenic cells were rapidly removed by the fixed tissue phagocytes of the spleen and liver. In a second communication Cary showed by extraction methods that organs rich in fixed tissue phagocytes are correspondingly rich in specific antibody content. He sees in his experiments confirmation of Kye's view that the fixed tissue phagocytes are active producers of antibodies. Motohashi confirmed Cary's findings for the spleen and liver, and showed that similar erythrocyte destruction occurred in the bone marrow. After splenectomy the reticulo-endothelial cells of the liver and especially of the bone marrow were found to be more active in ingesting foreign red blood-cells and in producing antibodies. If, however, the antigen was injected in large amount, the concentration of specific hemolysins was equal in normal and splenectomized animals. Bieling and Isaac found that splenectomy alone in mice had little effect on antibody formation, but if combined with injections of colloidal iron oxide (ferrum oxydatum saccharatum) to eliminate the activity of the remainder of the reticulo-endothelial system, the animals were unable to manufacture hemolysins. Siegmund had similar results. These experiments have also been confirmed for pneumococcus infections in mice (Neufeld and Meyer) and for typhoid agglutinins in rabbits (Koboyashi and Shuwotsu). Blocking the reticulo-endothelial system diminishes the immune response of tuberculous animals to tuberculin (Minami), and Freund noted that the development of hypersensitiveness could be further suppressed by combining injections of sugar of iron and trypan blue with splenectomy. It has been found possible to suppress antibody formation (hemolysins and precipitins) almost completely by injections of trypan blue alone (Gay and Clark, Isaacs) and to check the production of antitoxin in guinea pigs by large intravenous doses of India ink (Jungeblut and Berlot). The injections of dye and ink in these experiments did not seem to affect the general health of the animals.

Not all investigators, however, have succeeded in demonstrating reduced antibody production after attempts to "block" the reticulo-endothelial system and for this reason some have doubted that antibodies are formed in these cells. Rosenthal and his co-workers and Standenath

observed an increase in antibody concentration, whereas Weiss and Kunze found no change. Lewis and Loomis found that injections of trypan blue increase the capacity of the animal to react to antigenic substances. These contradictory experiments can be explained if it is assumed that the injections intended to block the reticulo-endothelial cells sometimes increase their activity. As Lewis and Loomis pertinently remark, it is a general rule that substances which exert physiological activity are stimulating in certain doses and depressing if their action is carried to the extreme.

Rats lose the power to store vital dyes in the reticulo-endothelial cells of the spleen when immunized against streptococcus vaccine (Paschkis), and rabbits immunized against pneumococci behave like non-immune animals after injections with India ink (Singer and Adler). Singer showed that injection of manganese chloride, a substance which is taken up by reticulo-endothelial cells, results in the appearance of agglutinins against OX19 in guinea pigs experimentally infected with typhus fever. In all probability the suppression of antibody formation in leukemia (Howell) can be attributed to the profound injury sustained by the reticulo-endothelial system in this disease.

The histiocytes appear to play an important rôle in local immunity processes as well. Portis found that the clasmatocytes of the omentum exhibited augmented activity of the height of antibody formation after intraperitoneal injections of antigen. Gay and Morrison noted that animals in which an exudate rich in polymorphonuclears was produced by means of aleuronat and other substances proved to be no more resistant than normal animals and frequently less so, whereas an abundance of histiocytes in the exudate insured the animal against many multiples of the fatal dose of streptococci. The observations of these authors led them to conclude that the local histiocytes are in large part if not entirely responsible for the natural resistance of rabbits to experimental streptococcus infections. Nakahara, extending these observations to other forms of bacterial infection in mice, found that the resistance of the peritoneum could be increased by provoking a macrophage reaction by means of preliminary intraperitoneal injections of olive oil.

Gay noted in animals immunized intrapleurally that with increasing grades of protection the subserous layer of the pleura, especially on the parietal and diaphragmatic surface, became greatly thickened, the original width increasing as much as forty times. This thickening was found to be due to infiltration of the subserosa with enormous numbers of mononuclear cells, a large proportion of which were shown to be his-

tiocytes by intrapleural injections of a vital dye (trypan blue). When bacteria were injected into the pleural cavity they were found after an hour or two within the histiocytes of the subserosa. After unilateral pleural immunization of advanced grade, the opposite pleura also acquired immunity. In this case the unprepared pleura remained histologically unaltered until injection of a test dose, following which the subserosa was found to be infiltrated with large numbers of histiocytes which could be shown to have been mobilized from their original site of proliferation on the immunized side. The older experiments of Carrel and Ingebrigtsen are worth mentioning in this connection. These investigators showed that hemolysin developed in vitro when antigen (goat's erythrocytes) was added to cultures of guinea pig's bone marrow or lymph nodes, tissues which are rich in reticulo-endothelial cells. A valuable contribution would be the demonstration of antibody production by a single strain of cells.

MISCELLANEOUS OBSERVATIONS ON THE ABSORPTIVE POWER OF THE RETICULO-ENDOTHELIAL SYSTEM. Saxl and Donath studied the effect of the intravenous administration of electrocollargol on the subsequent injection of water, dyes (phenoltetrachlorphthalein), drugs (adrenalin), and antiseptics (argochrome). Each of these substances was found to remain in the circulation longer than in the case of control injections without electrocollargol. These findings they attribute to functional "paralysis" of the reticulo-endothelial system, which normally appears to remove these substances from the circulation with great rapidity. These experiments suggest that the preliminary administration of electrocollargol or some similar substance might enhance the value of intravenous antiseptics by preventing their rapid removal from the circulation. Some therapeutic results which these authors obtained in infections seemed to be promising. In a subsequent communication, they reported that the reticulo-endothelial system is under pharmacologic control since urethane, morphia, atropine, and pituitrin diminish the absorption of an oil emulsion by these cells, whereas pilocarpin (in rabbits) reopens this system and allows the injected fat to be absorbed with greater rapidity, but these conclusions require confirmation.

Del Baere found that the disappearance of neosalvarsan from the circulation could be retarded by a preliminary injection of pituitrin. On the other hand, a primary injection of neosalvarsan appeared to "block" the reticulo-endothelial cells, retarding the subsequent absorption of neutral fat suspensions and congo red. These experiments support the opinion of Schlossberger that the reticulo-endothelial apparatus

is responsible for the rapid removal of neosalvarsan from the circulation when injected.[6]

Relation to certain clinical conditions. Chronic hemolytic jaundice, pernicious anemia, hemochromatosis, cirrhosis of the liver, thrombocytopenic purpura hemorrhagica, industrial disease due to radio-active substances.

In chronic hemolytic jaundice (congenital, familial or acquired) there is a conspicuous increase in bile pigment production, which can readily be demonstrated by estimating the bile pigment content of the stool or duodenal contents. Since hemoglobin is the only source of bilirubin, so far as we know at present, this finding would indicate increased destruction of erythrocytes. The excessive hemolysis is believed to occur mainly in the spleen, for removal of this organ results in disappearance of the jaundice, improvement of the anemia and general amelioration of the clinical condition. The improvement following splenectomy by no means signifies that the splenic changes represent the primary causative factor, which many believe to be the dysplasia of the erythrocytes which not only are smaller than normal in the average but exhibit increased fragility to hemolytic agents, e.g. hypotonic salt solutions. Both of these abnormalities, however, are usually ameliorated by splenectomy (Krumbhaar).

In many cases of pernicious anemia there is also augmented bilirubin production indicating excessive hemolysis. Hemosiderin is found not only in the reticulo-endothelial cells of the spleen, but also in the liver, lymph nodes and kidneys. The results of splenectomy are far less satisfactory than in hemolytic jaundice, the most that can be hoped for being temporary improvement. Peabody and Brown have recently called attention to the pronounced phagocytosis of red blood cells in the bone marrow in this disease. Doan employing the supra-vital technique found that the clasmatocytes in the bone marrow are greatly increased in number and that they phagocyte not only effete erythrocytes but also young immature nucleated forms which have not yet entered the circulation. His observations indicate that the spleen does

[6] Levin and Sacks found that phenolsulphonephthalein could not be recovered from the circulation 6 to 10 minutes after its intravenous administration, although the kidney continued to excrete it for 3 to 6 hours. Control experiments showed that the blood has no destructive effect on the dye. It appears probable that the dye is removed from the circulation in large part by the reticulo-endothelial cells especially of the liver and is slowly returned to the blood in small amounts for excretion by the kidneys.

not take an active part in the destruction of red blood cells in this disease.

The cause of the proliferation of the clasmatocytes in the bone marrow in pernicious anemia remains unknown. In this connection, reference may be made to the recent observations of Muller who showed that injections of collargol (colloidal silver) produced first a stimulating effect on the bone marrow with an outpouring of young erythrocytes and normoblasts, and later aplasia of the bone marrow with the collargol entirely in the endothelial cells (clasmatocytes) and a severe anemia resembling aplastic anemia. Since both the clasmatocytes and the erythrocytes are derived from the endothelial cells of the blood sinuses of the bone marrow (Doan, Cunningham and Sabin), the explanation of the results of these experiments appears to be that the collargol first stimulates the parent endothelial cell of the bone marrow to increased erythrocyte production but ultimately deviates the endothelial cell towards the formation of clasmatocytes at the expense of the development of erythrocytes. Elvidge noted similar results following the intravenous injection of finely divided quartz particles.

In pernicious anemia it appears that the entire hemolyto-poietic system is affected; the bone marrow which becomes hyperplastic attempts to compensate for the excessive erythrophagocytic activity of the hemolyto-poietic organs but fails in the end (Eppinger). While these observations may throw light on the pathological physiology of pernicious anemia the ultimate cause remains obscure.

In hemochromatosis there is a widespread deposition of hemosiderin and other pigments not only in the reticulo-endothelial cells of the liver, spleen, and lymph nodes but also in the skin, kidneys and all the important glands of the body. Cirrhosis of the liver and fibrosis of the pancreas are usually associated. Rous and Oliver have shown that chronic injections of blood in rabbits result in a widespread hemosiderosis almost identical with that characterizing human hemochromatosis. According to Mallory, there may be some relation between this disease and copper poisoning.

Eppinger believes that the reticulo-endothelial system is heavily implicated in cirrhosis of the liver. According to current views, especially in Europe, the entire structure of the liver in cirrhosis is disorganized as the result of extensive necrosis of the parenchyma and subsequent regeneration (*Umbau*), the normal lobular arrangement being completely effaced. Eppinger believes that the agent causing necrosis of the liver epithelial cells also destroys the Kupffer cells, those which

survive producing fibroblasts in great abundance, with consequent overgrowth of connective tissue, and that the reticulum cells of the spleen in turn initiate a similar overproduction of reticulum in that organ.

Splenectomy performed for the relief of idiopathic thrombocytopenic purpura hemorrhagica is generally curative (Kaznelson, Brill and Rosenthal, Giffin and Holloway, Alrutz, Nortell and Piette). The hemorrhages cease and the platelet count in most cases rises to normal. In certain cases the spleen is packed with platelets undergoing destruction by reticulo-endothelial cells. The results suggest that the spleen may normally be responsible for platelet destruction, but Krumbhaar states that the comparative platelet counts in the splenic artery and vein made by Holloway and Blackford fail to support this view. Perhaps the beneficial activity of splenectomy in some cases consists in stimulating more platelet formation. The existence of more than one type of thrombocytopenic purpura may explain some of the contradictory findings noted by various authors with relation to the rôle of the spleen and the number and quality of the platelets.

Martland, Conlon and Knef described a hitherto unrecognized form of occupational poisoning with necrosis of the jaw and severe leucopenic anemia resulting from the ingestion of luminous paint (zinc sulphide rendered luminous by radium and mesothorium). The necrosis results from local irritative radiation at the portal of entry, and the anemia appears to be due to the ingestion of radioactive elements with deposition of insoluble fixed particles in the cells of the reticulo-endothelial system, where they continually emit irritative rays which in time exhaust the hematopoietic centers. There is no known way of neutralizing or eliminating the fixed radioactive substances and there is danger of a fatal outcome from severe anemia years after their original deposition. The authors on the basis of their experience warn against the intravenous injections or internal administration of long-lived radioactive substances.

GENERAL SUMMARY

The reticulo-endothelial system, the cells of which were grouped together because of common phagocytic properties, reaction to vital dyes, and a general similarity of function, consists of the reticular and endothelial cells of the spleen, liver, bone marrow, lymph nodes (and lymphoid tissue generally) and the histiocytes of the general connective tissues. These are the cells which in the past have paraded under so many aliases and there is still no unanimity of opinion con-

cerning their nomenclature, origin and interrelationships. Recent investigations indicate that two types of histiocytes can be differentiated by means of the supra-vital technique, the monocyte and the clasmatocyte, the former being derived from a primitive reticular cell and the latter from endothelium. The transformations which the large mononuclear cells undergo in tissue cultures give rise to the impression that transitions between the monocytes and clasmatocytes may occur (Lewis and Lewis), but for the present these cells are best regarded as two distinct functional types (Sabin). The demonstration that the monocytes are derived from the same primitive reticular cell as the polymorphonuclear leucocytes and lymphocytes, and that the erythrocytes arise from the same endothelial cell (in the bone marrow) as the clasmatocytes would establish, if confirmed, a close relationship between the reticulo-endothelial system and the hematopoietic system.

Reticulo-endothelial cells of the hemolyto-poietic organs (spleen, liver, bone marrow, lymph nodes) may gain entry to the blood, in which they may be found constantly as monocytes as well as clasmatocytes. The potentialities of the large mononuclear cells of the blood are remarkable for their multiplicity and diversity, as illustrated by their power of transformation into macrophages capable of phagocyting red and white blood-cells, bacteria, pigment and other substances, their capacity to change hemoglobin into bilirubin, their ability to form fibroblasts, epithelioid cells and giant cells, and their faculty of metamorphosis into sarcoma-like tumor cells. Under certain pathological conditions, e.g. subacute bacterial endocarditis, typhoid fever, paroxysmal hemoglobinuria, these cells may be conspicuously increased in number and frequently exhibit highly phagocytic properties. To what extent these leukocytoses are monocytoses and to what extent clasmatocytoses must be established in future studies by means of the supra-vital technique.

The reticulo-endothelial system plays a predominant rôle in the specific infections. The epithelioid and giant cells of the tubercle are derived from reticulo-endothelial cells and, according to Sabin, Cunningham and their co-workers, from the monocytes alone. These monocytes can either emigrate from the blood to the site of tubercle formation or develop from local primitive reticular cells resident in the tissues. The reticulo-endothelial cells (histiocytes) are also the essential elements of the lesions in typhoid fever, the lepra nodule, the Aschoff bodies in rheumatic fever, and the specific lesions of actinomycosis, sporotrichosis, rhinoscleroma, Hodgkin's disease and other conditions.

In local inflammatory processes, these cells act as phagocytes of bacteria and scavengers to remove débris, and play an important rôle in the formation of granulation tissue. When bacteria are injected into the circulation they are quickly removed by the reticulo-endothelial cells. Their rôle in the production of immunity is a significant one, the available evidence indicating that antibodies are formed in these cells. In local infections, the degree of resistance depends in large measure upon the number of histiocytes in the exudate. The natural resistance of rabbits to experimental streptococcus infections can be best explained by the activity of these cells.

An important function of the reticulo-endothelial cells is the destruction of erythrocytes. There is constant fragmentation of red blood-cells in the circulation normally, and the fate of the fragments is to be taken up by endothelial cells both in the blood and in the hemolyto-poietic organs, especially the spleen. Under certain pathological conditions, e.g., hemolytic anemias, infections, etc., this rôle is greatly increased and the reticulo-endothelial cells are found laden with the products of the hemoglobin disintegration. When the spleen is removed the reticulo-endothelial cells of the other hemolyto-poietic organs quickly take over the spleen's share of blood destruction. The evidence is rapidly accumulating that the reticulo-endothelial system is the site of bile-pigment formation. Bilirubin is formed in significant amount in dehepatized dogs, proving that the liver epithelial cells are not essential for bile pigment production, and on the other hand it has been demonstrated that organs rich in reticulo-endothelial cells, e.g., spleen, bone marrow as well as the liver, *in vivo* as well as in tissue cultures, produce bilirubin from hemoglobin.

In hemolytic jaundice, splenectomy is often curative, indicating that the spleen is responsible for the excessive hemolysis which is characteristic of this disease. In pernicious anemia, on the other hand, the results of splenic extirpation are not satisfactory except as a temporary expedient, and this is possibly due to the excessive activity of other provinces of the reticulo-endothelial system, notably the bone marrow, in destroying red blood cells.

Disorders of the lipoid metabolism leave their mark on the reticulo-endothelial system. States of hypercholesterolemia either experimentally induced in rabbits, or in certain clinical conditions, notably diabetes and Niemann's disease, are associated with lipoid infiltration in the reticulo-endothelial cells, especially of the spleen. Gaucher's disease is a system disease of the reticulo-endothelial system which is

associated with a disturbance of the lipoid metabolism, but not of the hypercholesterolemic type, and leading to the deposition of a cerebroside-like substance and phosphatids in the Gaucher cells.

Histiocytic leukemias have been described, but suspected cases should in the future be studied by means of the supra-vital technique to discriminate between monocytic and clasmatocytic (endotheliocytic) forms. Leukemic-like proliferations of the reticulo-endothelial system but without leukemia occur, and various provinces may become the seat of primary new growth.

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